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(54) Title: T-TYPE VOLTAGE-GATED CALCIUM CHANNELS AND METHOD OF USING SAME (57) Abstract The present invention provides an isolated or substantially purified nucleic acid encoding a protein comprising at least one domain of a T-type calcium channel and cells and cell lines expressing such nucleic acids. The present invention also provides an isolated or substantially purified T-type calcium channel and an isolated or substantially purified antibody molecule recognizing an epitope on a T-type calcium channel protein.		

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T-TYPE VOLTAGE-GATED CALCIUM CHANNELS AND METHOD OF USING SAME

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This invention was made with Government support under Grant Number HL58728 awarded by the National Heart, Lung, and Blood Institute of the National Institutes of Health. The United States Government may have certain rights in this invention.

TECHNICAL FIELD OF THE INVENTION

The present invention relates to cloned T-type calcium channels.

BACKGROUND OF THE INVENTION

Biological membranes are themselves generally impermeable to ionic species. Thus, ions enter cells through regulated pores formed from membrane-associated proteins. Most of these regulated pores are voltage-dependent and are thus able to transduce changes in the transmembrane potential into ion flux. Voltage-gated ion channels form a "superfamily" of related proteins (cf. Jan et al., *Nature*, 345, 672 (1990)). Peculiar to this genus is a high degree of conservation in molecular structure. Generally, voltage-gated channels are membrane bound glycosylated proteins formed of many subunits. Large α subunits form a pore in the membrane that is selective for a given ionic species. Each α subunit contains four domains (I, II, III, and IV). Each channel domain has six putative transmembrane helical segments (S_1 - S_6). In general, the segments within each domain are similar but not identical. Aside from overall structural conservation, certain charged residues within the domains are highly conserved among voltage-gated ion channels (Jan et al., *supra*; Stühmer et al., *Nature*, 339, 597-603 (1989)).

Differences in charged residues between groups of voltage-gated ion channels confer properties unique to each subgroup, such as ion selectivity. For example, most voltage gated ion channels are selective for either sodium, potassium or calcium. Known calcium channels require a ring of negative charge provided by glutamate residues found at similar locations in each of the domains (Yang et al., *Nature*, 366, 158-61 (1993)).

Voltage-gated channels are often classified on the basis of their electrophysiology. The resting membrane potential of most animal cells is between about -70 mV and -80 mV. When the membrane becomes depolarized (moved towards 0 mV), various membrane channels become activated (they are said to

“open”). Thus, one basis for classifying membrane channels is the membrane potential necessary to activate (or “gate”) them (voltage dependency). For example, “T-type” calcium channels are activated at a lower voltage than L- or N-type channels (Nowycky et al., *Nature*, 316, 440-43 (1985)). Other physiological properties are the activation kinetics, inactivation kinetics, tail current (deactivation kinetics), and single channel conductance. Thus, in comparison to other calcium currents, T-type calcium current is characteristically short (Chen et al., *J. Gen. Physiol.*, 96, 603-30 (1990)), and it exhibits characteristically slow activation kinetics near threshold, fast inactivation kinetics, and slow tail current (Randall et al., *Neuropharmacol.*, 63, 879-93 (1997); Carbone et al., *Nature*, 310, 501-02 (1984); Nilius et al., *Nature*, 316, 443-46 (1985)).

Calcium currents have been implicated in many neurological and muscular functions. For example, T-type calcium current is associated with cardiac pacemaker activity, pain transmission in the central nervous system, and in other physiological functions. Defects in T-type calcium current have been implicated in cardiac arrhythmia, hypertension, and epilepsy. Given their potential clinical value, the pharmacological properties of calcium channels have been the subject of extensive study. Most such studies have involved L-type channels because, unlike T-type channels, L-type calcium channels are readily purified from cell extracts. For example, L-type calcium channels have been purified using dihydropyridine drugs (e.g., nifedipine) which can bind with sufficiently high affinity to serve as a ligand for purifying L-type calcium channels. Such purified and cloned L-type calcium channels have been used to develop assays for drugs affecting L-type calcium channels (see, e.g., U.S. Patents 5,429,921 and 5,386,025).

While many electrophysiological characteristics of T-type calcium currents are known, the lack of isolated T-type channels has stalled research into the pharmacology and biophysics underlying the T-type calcium current, at least in comparison with other calcium channels. Indeed, while it is generally assumed that voltage-sensitive ion channels are responsible for the current, no such channel protein, nor any nucleic acid encoding such a protein, has been isolated. In view of the foregoing problems, there exists a need for an isolated T-type calcium channel and a nucleic acid encoding a T-type calcium channel.

BRIEF SUMMARY OF THE INVENTION

The present invention provides an isolated or substantially purified nucleic acid encoding a protein comprising at least one domain of a T-type calcium channel and cells and cell lines expressing such nucleic acids. The present invention also provides an isolated or substantially purified T-type calcium channel and an isolated or

substantially purified antibody molecule recognizing an epitope on a T-type calcium channel protein.

The present invention is useful for exploring the electrophysiology and pharmacology of the T-type calcium current. Such knowledge can lead to the development of drugs for potentiating or attenuating T-type calcium channels. Thus, the present invention provides an assay for identifying potential drugs affecting T-type calcium channels by exposing cells expressing a T-type calcium channel to a putative drug and then measuring the calcium flux in response to a change in membrane potential. The identification of drugs affecting T-type calcium channels will facilitate even greater understanding of the biophysics of these proteins. Furthermore, some such drugs could have potential clinical applications.

The invention can best be understood with reference to the accompanying drawings and in the following detailed description of the preferred embodiments.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1A-1E compare the complete amino acid sequences of three types of T-type calcium channels ($\alpha 1G$ (or $Ca_vT.1$), $\alpha 1H$ (or $Ca_vT.2$), and $\alpha 1I$ (or $Ca_vT.3$)), indicating conserved functional domains.

Figures 2A-2D are graphic representations of the current-voltage relationships of three cloned T-type calcium channels (Figures 2A, 2B, and 2C) and a cloned R-type calcium channel (Figure 2D).

Figure 3A is a graphic representation of the average current-voltage curve for cloned T-type calcium channels ($\alpha 1G$, triangles, $\alpha 1H$, inverted triangles, $\alpha 1I$, circles), and a cloned R-type calcium channel (filled squares). Figure 3B compares the normalized conductance of a cloned T-type calcium channel at three different concentrations of $BaCl_2$.

Figure 4 depicts average kinetics of the tail current as a function of repolarization potential for $\alpha 1G$ (triangles), $\alpha 1H$ (inverted triangles), $\alpha 1I$ (circles), and a cloned R-type calcium channel (filled squares).

Figures 5A and 5B graphically present data concerning the use of a cloned T-type calcium channel to detect drugs affecting the channel. Figure 6A depicts the effect of 100 μM on current-voltage relationships with a single dosage of mibefradil. Figure 6B illustrates the effect on T-type channel conductance of various doses of mibefradil.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present invention provides an isolated or substantially purified nucleic acid encoding a protein comprising at least one domain of a T-type calcium channel α

subunit. The nucleic acid can be of any type, and it can include other elements aside from a sequence encoding a T-type calcium channel domain or domains. For example, where the nucleic acid comprises RNA, it can also include regulatory sequences suitable to permit translation of the RNA. Thus, an RNA nucleic acid of the present invention preferably has at least one ribosome entry site, and preferably has a polyadenosine tail for stabilizing the RNA in the cellular environment.

Similarly, DNA nucleic acids of the present invention can have regulatory elements for promoting the transcription of sequence encoding the T-type calcium channel into an RNA such as that described above. For example, a DNA nucleic acid of the present invention can have a promoter and/or an enhancer sequence. While the nucleic acid can be any type of nucleic acid, the nucleic acid preferably comprises a cDNA. A cDNA nucleic acid is preferred over other nucleic acids to permit the nucleic acid to be readily cloned, sequenced, and expressed in a wide variety of cells.

The choice of promoter and/or an enhancer will largely depend on the milieu in which the nucleic acid is to be expressed. Thus, for expression in bacterial cells, the regulatory elements are bacterial promoters. Similarly, for expression in mammalian cells, the regulatory elements are able to effect expression in mammalian cells. While many such regulatory elements are known in the art, examples include prokaryotic promoters and viral promoters (e.g., retroviral ITRs, LTRs, immediate early viral promoters (IEp), such as herpesvirus IEp (e.g., ICP4-IEp and ICP0-IEp), cytomegalovirus (CMV) IEp, and other viral promoters, such as Rous Sarcoma Virus (RSV) promoters, and Murine Leukemia Virus (MLV) promoters). Other suitable promoters are eukaryotic promoters, such as enhancers (e.g., the rabbit β -globin regulatory elements), constitutively active promoters (e.g., the β -actin promoter, etc.), signal specific promoters (e.g., inducible promoters such as a promoter responsive to RU486, etc.), and tissue-specific promoters (e.g., those active in epidermal tissue, dermal tissue, tissue of the digestive organs (e.g., cells of the esophagus, stomach, intestines, colon, etc., or their related glands), smooth muscles, such as vascular smooth muscles, cardiac muscles, skeletal muscles, lung tissue, hepatocytes, lymphocytes, endothelial cells, sclerocytes, kidney cells, glandular cells (e.g., those in the thymus, ovaries, testicles, pancreas, adrenals, pituitary, etc.), tumor cells, cells in connective tissue, cells in the central nervous system (e.g., neurons, neuralgia, etc.), cells in the peripheral nervous system, and other cells of interest).

The isolated or substantially purified nucleic acid of the present invention encodes all or part of a T-type calcium channel α subunit. As used herein, a "calcium channel" includes a protein structure for facilitating the flux of calcium ions across a biological membrane into which the calcium channel is inserted. As used herein, a "T-type channel" is a type of voltage-gated ion channel that facilitates the flux of ions

when the membrane potential of a biological membrane into which it is inserted experiences a slight depolarization. Thus, a T-type calcium channel can begin to gate from about -60 mV to about -30 mV (i.e., about -45 mV to about -35 mV) in about 10 mM Ba^{2+} . Additionally, T-type channels of the present invention exhibit a slow
5 deactivation (tail current) following depolarization. Thus, a T-type calcium channel can exhibit a tail current that decays exponentially with a tau value from about 1 ms to about 10 ms (e.g., from about 4 ms to about 7 ms, such as about 6 ms) following repolarization to a membrane potential from about -80 mV to about -60 mV in a solution with a Ba^{2+} concentration of from about 10 mM to about 40 mM. Another
10 defining characteristic of T-type calcium channels is that they exhibit small single channel conductance. Thus, for example, a T-type channel exhibits a single channel conductance of from about 4 pS to about 12 pS (e.g., from about 6 pS to about 10 pS), and typically from about 7 pS to about 9 pS in a solution with a Ba^{2+} concentration of about 0.1 M.

15 The isolated or substantially purified nucleic acid of the present invention encodes all or part of any T-type calcium channel having at least one of the aforementioned electrophysiological properties when properly assembled within a cellular membrane. The general structure of calcium channels is summarized above and is otherwise known in the art. Thus, for example, the nucleic acid can encode one
20 of the four functional domains mentioned above. As used herein, a domain of a T-type calcium channel is any protein structure able to associate with three other domains to form a tetrameric body functioning as a T-type calcium channel. While the native T-type calcium channel structure includes all four domains in a single polypeptide (indicated in Figures 1A-1E), a domain can exist as a polypeptide species
25 separate from those containing the other domains. Such separate domains are able to associate within the plasma membrane to form a functional channel. Alternatively, where a plurality of domains are linked within a common polypeptide, the linkage can deviate substantially from the native linkage. Thus, for example, the domains can be linked by polypeptide sequences other than those sequences linking the domains in the
30 native protein (e.g., non-native polyglutamate linkages). Indeed, the domains themselves can include non-native linkages between membrane-spanning elements within the domains. Aside from these modifications, the nucleic acid can encode a chimeric calcium channel domain (or an entire channel) comprising a portion of a T-type calcium channel and a portion derived from another calcium channel (or other
35 channel) protein. For example, the chimera can include portions of domains from T-type channels responsible for low voltage gating and portions of domains from other calcium channels responsible for slow inactivation. Such a protein exhibiting T-type gating but longer inactivation kinetics would facilitate pharmacological research.

As mentioned, nucleic acids of the present invention can encode an entire T-type channel (i.e., a T-type channel protein comprising four functional domains). It has been discovered that at least three genes encoding T-type calcium channels exist in humans and rats (i.e., $\alpha 1G$ (or Ca_vT.1), $\alpha 1H$ (or Ca_vT.2), and $\alpha 1I$ (or Ca_vT.3)), and alternate splicing of these isoforms exist. Examples of the amino acid sequences of full-length T-type channels, and the sequences of suitable coding nucleic acids are set forth at SEQ ID NOs:1-8 ($\alpha 1G$ sequences), SEQ IS NOs:9-10 ($\alpha 1H$ sequences), and SEQ ID NOs: 11-12 ($\alpha 1I$ sequences). However, the invention is not limited to these exemplary sequences. Indeed, as mentioned, an amino acid sequence of a T-type calcium channel can vary from those listed, and it is within the state of the art to change a nucleotide sequence encoding a T-type channel to introduce mutations into the protein. Indeed, for conducting electrophysiological assays, it may be desirable to introduce mutations into such a protein. For example, mutations comprising insertions or deletions can be introduced on either the amino- or carboxy-terminus of the protein, or such mutations can be intrasequence insertions or deletions. Where the electrophysiological properties of the calcium channel are to be conserved, such mutations preferably are in regions other than the membrane spanning domains. However, in some applications (e.g., to decrease inactivation kinetics), the changes can be within the membrane-spanning regions. Moreover, as mentioned above, the sequence can form a protein having only one functional domain of a T-type calcium channel. Additionally, the sequence can also form a chimeric protein or domain, such as those described above.

Aside from insertions and deletion mutations of native T-type calcium channel sequences, a T-type calcium channel can include substitutions of amino acid residues, e.g., for those indicated in SEQ ID NOs:1-12. Preferably, and especially where such a substitution is within a membrane spanning region, the substitution is conservative. Thus, within membrane spanning domains, positively-charged residues (H, K, and R) preferably are only substituted with positively-charged residues; negatively-charged residues (D and E) preferably are only substituted with negatively-charged residues; neutral polar residues (C, G, N, Q, S, T, and Y) preferably are only substituted with neutral polar residues; and neutral non-polar residues (A, F, I, L, M, P, V, and W) preferably are only substituted with neutral non-polar residues. Preferably, any amino-acid substitution within the membrane-spanning regions does not alter this conservation. Most preferably, any substitution, deletion, or insertion does not alter the IVS4 domain. In each of the exemplary T-type calcium channel α subunit sequences, the putative IVS4 region comprises SEQ ID NO:13. Given the strong sequence conservation among families of voltage-gated ion channels, it is likely that this sequence or a derivative sequence, will be present in T-type channels. Thus, the

present invention provides any T-type calcium channel (or a nucleic acid encoding such a T-type calcium channel) comprising SEQ ID NO:13 or a sequence derived from SEQ ID NO:13 having conservative amino acid substitutions, as described above.

5 The nucleic acid of the present invention encoding all or a part of a T-type calcium channel can be isolated via any suitable method. For example, prior to the present invention, one of skill in the art could design a probe based on the sequence of known, non-T-type, calcium channels and use such probe to screen a genetic library. If such a screen were to identify a putative calcium channel, the researcher could then
10 attempt to clone the entire nucleic acid to characterize it. Similarly, prior to the present invention, to isolate a nucleic acid encoding a T-type calcium channel, one of skill in the art could consult publicly available databases containing DNA sequences (e.g., Genbank) to locate nucleic or amino acid sequences representing a portion of a T-type calcium channel protein or nucleic acid. However, such databases contain no
15 sequence for a full-length T-type calcium channel or identify any sequence as a T-type channel. Such methods assume that T-type calcium channels share sufficient sequence identity with known calcium channel nucleic acids to cross-hybridize, an assumption not supported by any published report. Moreover, prior to the present invention, no partial sequence in such databases was identified as corresponding to a
20 T-type calcium channel. Thus, prior to the present invention, the presence of partial sequences in the public DNA databases could facilitate the isolation of T-type calcium channels only with the exercise of a considerable degree of speculation on the part of the researcher.

By providing several sequences pertaining to T-type calcium channels and a
25 comparison presenting conserved regions and domains, the present invention greatly facilitates the isolation of other nucleic acids encoding T-type calcium channels (or derivatives thereof) with much less experimentation. Thus, while any of the methods discussed above can be employed to isolate other members of this genus, preferably, a nucleic acid encoding a T-type calcium channel is isolated by probing a genetic library
30 using a probe that hybridizes to a DNA encoding a peptide sequence contained in (or similar to) a known T-type calcium channel (e.g., SEQ ID NOs:1-12). To facilitate the isolation of a T-type calcium channel, the present invention provides an isolated polynucleotide hybridizing to a portion of the nucleic acid of the present invention encoding a T-type calcium channel (or a portion thereof). Thus, for example, the
35 present invention includes an isolated polynucleotide hybridizing to SEQ ID NO:1-12. The isolated polynucleotide can hybridize to all or any portion of the sequence encoding the T-type calcium channel.

To isolate such a polynucleotide, any portion of a sequence encoding a T-type calcium channel can be employed as a probe to screen a genetic library, and such screening can be accomplished by standard techniques known in the art. While the probe can hybridize to any portion of such a DNA, preferably the probe is designed to hybridize to a DNA encoding a polypeptide sequence that is highly conserved among T-type calcium channels but is less conserved between the genus of T-type calcium channels and other proteins. Such peptide sequences are readily apparent from the sequence comparison set forth in Figures 1A-1E. Generally, the specificity of hybridization in a genetic screen varies depending on the length of the probe and the stringency (e.g., temperature, salt and detergent concentration, etc.) of hybridization. Stringency of hybridization is broadly classified as "high," "moderate," or "low," and the parameters of these terms are well recognized in the art (see, e.g., Sambrook et al., "Molecular Cloning, a Laboratory Manual," Cold Spring Harbor Press, 1989). The isolated polynucleotide hybridizing to a portion of the nucleic acid encoding a T-type calcium channel can hybridize under any desired stringency conditions. However, for identifying other T-type channels, preferably, the hybridization occurs under moderate stringency, and most preferably under high stringency.

Of course, the isolated or substantially purified polynucleotide can itself be employed as a probe to screen a library as described to isolate a second nucleic acid. In such a screen, one of the polynucleotides will be complementary to a portion of the sequence encoding the T-type calcium channel, and the other isolated nucleic acid will be "sense." Preferably, one of the two isolated polynucleotides (the "sense" strand) itself encodes a T-type calcium channel, or at least one domain thereof. Such a sequence can be cloned to be operably linked to suitable regulatory elements, as described, to produce a T-type calcium channel. Thus, aside from using the nucleic acid of the present invention to produce a T-type calcium channel, the nucleic acids of the present invention are also useful for isolating other sequences encoding T-type calcium channels, or derivatives thereof.

However isolated, the isolated or substantially purified nucleic acid of the present invention is useful, in part, for producing all or a portion of a T-type calcium channel. Thus, the nucleic acid can be introduced into a suitable milieu for driving its expression. Because T-type channels are transmembrane proteins, preferably such a milieu is a living cell. However, it should be understood that the nucleic acid can also be expressed *in vitro* under conditions, such as those known in the art, suitable for *in vitro* transcription and translation. However produced, the present invention includes any protein, such as a recombinant protein or an isolated or substantially purified protein, including all or a portion of a T-type calcium channel or a protein derived from a T-type calcium channel.

For expression in a living cell, the nucleic acid must be introduced into the cell. As nucleic acids are generally introduced into cells as part of genetic vectors, the present invention provides a vector having a T-type calcium channel nucleic acid of the type described above. Any type of vector suitable for introducing the nucleic acid into a host cell is within the context of the present invention. Examples of such vectors include naked DNA and RNA vectors (such as oligonucleotides, plasmids, capped cRNA, etc.), viral vectors such as adeno-associated viral vectors (Berns et al., *Annals of the New York Academy of Sciences*, 772, 95-104 (1995)), adenoviral vectors (Bain et al., *Gene Therapy*, 1, S68 (1994)), herpesvirus vectors (Fink et al., *Ann. Rev. Neurosci.*, 19, 265-87 (1996)), packaged amplicons (Federoff et al., *Proc. Nat. Acad. Sci. USA*, 89, 1636-40 (1992)), papilloma virus vectors, picornavirus vectors, polyoma virus vectors, retroviral vectors, SV40 viral vectors, vaccinia virus vectors, and other vectors. Once a given type of vector is selected, its genome must be manipulated for use as a background vector, after which it must be engineered to incorporate exogenous polynucleotides. Such manipulations are known in the art.

The vectors of the present invention are useful for introducing a nucleic acid encoding all or a portion of a T-type calcium channel into a host cell. Thus, the present invention provides a cell into which the vector of the present invention has been introduced. The host cell can be any cell suitable for expressing the nucleic acid (e.g., bacteria, insect cells, mammalian cells, etc.). The host cell can thus be *in vitro* or *in vivo*. Preferably the cells do not exhibit native T-type calcium current. A preferred cell type is HEK-293 cells because they contain genetic elements that facilitate the expression of transgenes from a variety of expression vectors. For facilitating electrophysiological recordings, oocytes (e.g., *Xenopus* oocytes) are preferred, as they are large and readily handled.

The vector can be introduced into the cell in any manner suitable for the cell type and vector employed. In one embodiment, the vector can be used to prepare an RNA transcript *in vitro* (e.g., a capped cRNA) which is then introduced into the host cell by standard methods (such as injection). Such techniques are preferred when the host cells do not actively transcribe DNA (such as oocytes). In other embodiments, a DNA vector is introduced into the cell such that it is transcribed within the cell. For example, the vector can be introduced into the cell such that it forms an extrachromosomal segment of genetic material in the cell, as is the case with many types of viral vectors. Alternatively, the vector can introduce the nucleic acid into the chromosomal DNA of the host cell.

Preferably, a cell into which the nucleic acid is introduced is also able to express the nucleic acid to produce the α subunit protein. The expression of the nucleic acid can be detected by probing the cell for the presence of T-type calcium

channel mRNA, such as via Northern hybridization analysis, in situ hybridization, etc. More preferably, however, the cell is able to express the nucleic acid to produce the protein including all or a portion of a T-type calcium channel. In such cells, expression of the nucleic acid is confirmed by detecting the protein, for example, by
5 probing cellular extracts with an antibody recognizing the protein (e.g., on a Western blot, etc.).

In the membrane of the cell producing the protein, the expressed protein contributes to the formation of a functional calcium channel. Where the protein encodes an entire α subunit, the full protein will possess some or all of the
10 electrophysiological properties of T-type calcium channels described above. Where the protein encodes less than an entire channel α subunit (e.g., a domain), the protein will aggregate with other constituent domains in the membrane to form a functional channel. Thus, the presence of the protein can be detected by assaying the cell for T-type calcium channel activity. Indeed, assaying for channel activity serves to
15 determine whether a nucleic acid encoding a putative calcium channel, in fact, encodes a species of T-type channel (as opposed to a member of another genus of calcium channels). For example, when large cells (e.g., oocytes) are used as the host cells, the electrophysiological properties of the channel can be investigated. Thus, the membrane activity of whole cells expressing the nucleic acid can be measured
20 directly, such as via patch clamp techniques using a voltage clamp electrode and a current electrode (Bernal et al., *J. Pharmacol. Exp. Ther.*, 282, 172-80 (1997)). Alternatively, the activity of single channels can be measured, such as with a standard depolarizing bath and pipette solutions (Lacerda et al., *Biophys. J.*, 66, 183-43 (1994)). However measured, the properties of cells into which the putative nucleic
25 acid is introduced are compared to the channel conductance, voltage dependency, activation kinetics, inactivation kinetics, or tail current known for T-type channels and discussed above. A measure of current density (e.g., pA/pF) can also be used to assess the level of gene expression in the cells, normalizing for cellular volume.

While, in accordance with the present invention, an isolated cell into which the
30 T-type calcium channel nucleic acid has been introduced (and preferably stably expressing the nucleic acid to produce the protein) can be prepared, preferably, such transfection protocols result in a population consisting essentially of such transfected cells. For standardizing the results of many experiments, it is even more desirable to employ an established cell line consisting essentially of such cells. Preferably, for use
35 in high throughput assays, cell lines stably expressing a T-type calcium channel exhibit a current density of at least about 40 pA/pF (e.g., at least about 45 pA/pF), such as about 50 pA/pF or even 55 pA/pF or higher. Preferably, a cell line in accordance with the present invention is able to propagate the nucleic acid through

several passages (e.g., for at least 10 passages), and, preferably, the nucleic acid is stably integrated into the chromosomes of such cells. Thus, the cell line can propagate the nucleic acid for at least 20 passages, and more preferably significantly more than 20 passages (e.g., at least about 25 passages, or even more).

5 Regardless of the cell system, the ability to express a T-type calcium channel nucleic acid within host cells to produce an active channel permits the channel to be further studied. In this regard, the present invention provides a method of identifying a drug which affects T-type calcium channels. The method involves first expressing a T-type calcium channel in a cell to produce an active channel, as herein described.

10 The cell expressing the channel is then exposed to a solution containing a putative drug for interfering with the channel. Thereafter, the presence or absence of calcium flux in response to a change in membrane potential is assayed. Any such assay can be employed within the context of the present invention, (e.g., using labile dyes, radioisotopes (e.g., ^{45}Ca), recording electrophysiological changes in the membrane, etc.). A quick method of assaying for calcium flux is first to introduce a
15 calcium-sensitive labile dye into the cells. For example, the dye can be one such as those that fluoresce or change color in the presence of calcium, many of which are known to those of skill in the art (e.g., Indo-1). Thereafter, the cells are exposed to a depolarizing solution containing high (e.g., about 50 mM) potassium concentration
20 and a drug, and the reaction of the labile dye is compared to control cells. Using a labile dye affords the ability to assay many putative drugs quickly in a high throughput assay for putative drugs affecting T-type channels. For example, the initial screening can be carried out in 96 well plates. Moreover, dose-response data can be readily generated by exposing the cells to several concentrations of the same putative
25 drug.

 Once a putative drug is detected, its effect on the electrophysiology of the cell (e.g., single channel conductance, voltage dependency, activation kinetics, inactivation kinetics, and tail current of the cells) can be investigated in detail.

30 Generally, the effect of the putative drug on T-type calcium currents is assessed by measuring the various electrophysiological parameters in the presence of various concentrations of the drugs and comparing the data to untreated (or sham-treated) control cells. Cells preferably are maintained in a continuous perfusion chamber during such experiments to facilitate changing solutions. The inventive method of identifying a drug which affects T-type calcium channels can employ any nucleic acid
35 encoding a T-type calcium channel (or derivative thereof), such as those nucleic acids described herein. In fact, as several isoforms of T-type channel exist, the assay method can be repeated using nucleic acids encoding different isoforms to identify

drugs that preferentially target a given isoform, or drugs which affect more than one isoform of T-type calcium channels.

Aside from affording an *in vitro* assay for detecting potential therapeutic or investigative drugs targeting T-type calcium channels, the method of expressing the T-type calcium channel nucleic acid can also be used *in vivo*. For example, as mentioned, several neurological and muscular diseases or disorders have implicated mutations affecting native nucleic acids encoding T-type calcium channels. The present invention, thus, provides a method of treating a disease or disorder associated with a deficiency in a native T-type calcium channel nucleic acid. The method involves introducing a vector having the T-type calcium channel nucleic acid into cells of a host in which native expression of the nucleic acid is deficient. Thus, for example, for treating cardiomyopathy associated with deficiencies in T-type calcium channels, the vector is introduced into myocardial cells. Similarly, for treating forms of epilepsy associated with deficiencies in T-type calcium channels, the vector is introduced into neurons (e.g., thalamic neurons). Within the target cells, the nucleic acid within the vector is expressed to produce active T-type calcium channel. By similar methods, an nucleic acid having a sequence antisense to a sequence encoding a T-type calcium channel (or a portion thereof) can be expressed within a cell. The presence of an antisense sequence can down-regulate the expression of native T-type calcium channel genes by hybridizing to T-type channel mRNA within the cell. Thus, the present invention is useful to treating disorders associated with over-expression of T-type calcium channels.

T-type channel proteins (such as whole T-type calcium channels, domains of such channels, chimeras including portions of T-type calcium channels, etc.) can be employed to generate antibodies (e.g., immunoglobulins) to T-type calcium channels. Thus, the present invention provides an isolated and substantially purified antibody molecule recognizing an epitope on a T-type calcium channel. Such antibodies can be monoclonal antibodies or polyclonal antisera. Antibodies recognizing T-type calcium channels can be used to purify the channels from cell extracts or other solutions by standard methodologies (e.g., immunoprecipitation). Moreover, depending on the location of the epitopes for the antibodies on the T-type calcium channel, the antibodies can be used to affect the channel proteins present on the surface of cells. Thus, antibodies directed to T-type calcium channels are potential reagents for studying the channels as well as for therapy.

Such antibodies can be produced by any suitable method, many of which are well known in the art. Thus, for example, the antibodies can comprise polyclonal antisera obtained from inoculated animals. Alternatively, the antibody molecules can be monoclonal antibodies obtained from a cell line (e.g., a hybridoma cell line). Thus,

the present invention provides a cell which produces such antibodies. Such a cell can be *in vitro* or *in vivo*; however, where the cell is *in vitro*, preferably it is within an established cell line consisting essentially of such cells.

Several examples are presented below to illustrate the invention. Taken together, the examples demonstrate the cloning of twelve novel proteins and their characterization as T-type calcium channel α subunits. These examples are included here for purely illustrative purposes; as such, they are not to be construed so as to limit the scope of any aspect of the invention.

Many procedures employed in the following examples are techniques routinely performed by one of ordinary skill in the art (see generally Sambrook et al., *Molecular Cloning. A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (1989)) and are not discussed in detail. However, some reagents and methods deserve specific description. Thus, for example, *in vitro* translation and expression were conducted as described previously (Schneider et al., *Receptors and Channels*, 2, 255-70 (1995)). *Xenopus laevis* oocytes were prepared as described previously (Bernal et al., *J. Pharmacol. Exp. Ther.*, 282, 172-80 (1997)). To express proteins, 10 or 30 ng of capped cRNA was injected into the oocytes in a volume of 50 nl. For single channel recording, oocytes were injected with 100 ng capped cRNA and incubated for one week prior to assay.

Cells were voltage clamped using a two-microelectrode voltage clamp amplifier as described (Bernal et al., *J. Pharmacol. Exp. Ther.*, 282, 172-80 (1997)). The standard bath solution contained the following: 40 mM Ba(OH)₂, 50 mM NaOH, 1 mM KOH, 0.1 mM EDTA, and 5 mM HEPES, adjusted to pH 7.4 with methanesulfonate. The osmolality of the 2 mM Ba²⁺ and 10 mM Ba²⁺ solutions was balanced by increasing the NaOH concentration as described (Lory et al., *J. Physiol.*, (London), 429, 95-112 (1990)). Voltage and current electrodes (1.5-1.8 M tip resistance) were filled with 3 M KCl. Except as noted, data were acquired at 4 kHz using the pCLAMP system, and filtered at 1 kHz. Data were analyzed using pCLAMP software. Boltzman fits and linear regression were calculated using Prism.

EXAMPLE 1

This example demonstrates the cloning and characterization of putative T-type calcium channels.

A search of the Genbank library was conducted to identify clones identified as having some degree of homology to known calcium channel sequences. The search identified an expressed sequence tagged (EST) partial sequence in a human brain clone (H06096), which was used as a probe to screen a λ gt10 cDNA library prepared

from rat brain. Successive screening of the cDNA library identified five overlapping clones which were aligned to construct an entire cDNA sequence, termed $\alpha 1G$.

The $\alpha 1G$ cDNA was cloned into the pSP72TM vector and sequenced by standard computer-assisted sequencing. Using the $\alpha 1G$ cDNA, the amino acid sequence of the $\alpha 1G$ protein was deduced and compared to the sequences of other known calcium channel α subunits. By similar methods, homologous human (H19230 and R19524) and mouse (AA286626) EST clones were also identified and partially sequenced, and alternately spliced variants were identified. The deduced cDNA and amino acid sequences for eight full-length $\alpha 1G$ T-type channels are set forth, respectively, as SEQ ID NOs:1-8.

A second T-type calcium channel, termed $\alpha 1H$, was isolated by screening a human heart cDNA library with a fragment of the $\alpha 1G$ sequence. An alternately spliced isoform was also identified. The full-length cDNA and amino acid sequences for these $\alpha 1H$ T-type channels are set forth, respectively, as SEQ ID NOs:9 and 10.

A third T-type calcium channel, termed $\alpha 1I$, was isolated by screening a rat brain cDNA library at low stringency using a fragment of the rat $\alpha 1G$ gene. Fifty plaques were identified, many of which were not detected in a second screening. A third screening with a fragment from $\alpha 1H$ identified two clones. Subsequent screening, and the use of the GenBank database, led to the identification of the full length rat and human cDNA and amino acid sequences, set forth at SEQ ID NOs: 11 and 12, respectively.

The $\alpha 1G$, $\alpha 1H$, and $\alpha 1I$ amino acid sequences were compared to each other and a known calcium channel ($\alpha 1E$) to investigate the conservation of protein structure and function. The comparison indicates that the $\alpha 1G$, $\alpha 1H$, and $\alpha 1I$ amino acid sequences within the putative membrane-spanning domains are about 90 % identical to each other, while the $\alpha 1G$, $\alpha 1H$, and $\alpha 1I$ sequences are only roughly 40 % identical to the $\alpha 1E$ clone.

Figures 1A-1E indicate this conservation between the proteins. The conservation of charged residues, particularly in the S4 domains, is consistent with the role of the $\alpha 1G$, $\alpha 1H$, and $\alpha 1I$ proteins as ion channels. However, two of the glutamates associated with ion specificity in other calcium channels have been replaced with aspartate, suggesting altered ion selectivity. Strikingly, $\alpha 1G$, $\alpha 1H$, and $\alpha 1I$ display only low homology to sequences linking the membrane-spanning regions within each domain, and even less homology between the intracellular loops linking domains. Notably, neither $\alpha 1G$, $\alpha 1H$, nor $\alpha 1I$ possesses sequences known to bind β subunits or Ca^{2+} ions.

EXAMPLE 2

This example demonstrates the production of cell lines stably expressing the cloned $\alpha 1G$, $\alpha 1H$, and $\alpha 1I$ proteins.

HEK-293 cells were transfected with either the rat $\alpha 1G$ cDNA (SEQ ID NO:1), the human $\alpha 1H$ cDNA (SEQ ID NO:9), or the rat $\alpha 1I$ cDNA (SEQ ID NO:11). As a control, cells were also transfected with human $\alpha 1E$ plus human $\beta 3$ (Schneider et al., *Receptors Channels*, 2, 255-70 (1994); Murakami et al., *Eur. J. Biochem.*, 236, 138-43 (1996)). The DNA constructs included a neomycin resistance gene conferring resistance to G418. The cells were cultured under standard conditions using medium containing G418 to select for stable transformants.

Surviving clones were expanded and assayed for electrophysiological activity to determine the presence of channels within the membrane. Whole-cell currents were recorded from ruptured patches using an Axopatch 200A amplifier, Digidata 1200 A/D converter, and pCLAMP 6.0 software. Data were digitized at 2 kHz and filtered at 1 kHz or off-line. All experiments were performed at room temperature. Pipettes were made out of TW-150-6 capillary tubing (World Precision Instruments, Inc., Sarasota, FL), using a Model P-97 Flaming-Brown pipette puller (Sutter Instrument Co., Novato, CA). The internal pipette solution contained the following: 55 mM CsCl, 75 mM CsSO₄, 10 mM MgCl₂, 0.1 mM EGTA, 10 mM HEPES, pH adjusted to 7.2 with CsOH. The external Tyrodes solution was the following: 140 mM NaCl, 6 mM KCl, 2 mM CaCl₂, 10 mM glucose, 5 mM HEPES, pH 7.4. The recording solution contained the following: 10 mM BaCl₂ solution (or 2 mM CaCl₂), 140 mM tetraethylammonium (TEA) chloride, 5 mM CsCl, 1 mM MgCl₂, 5 mM glucose, and 10 mM HEPES, pH adjusted to 7.4 with TEA-OH. Under these solution conditions the pipette resistance was typically 1.5-2.5 M Ω . Cell capacitance was measured by integrating the charging current during a 10 mV hyperpolarizing pulse (holding potential -80 mV).

Using these recording techniques, values for pA/pF were obtained for each cell line, which is a measure of current density normalizing for cell size. One clone (#N2) expressed the rat $\alpha 1G$ protein and has a current density of 42 pA/pF. Another clone (#13), expressed the human $\alpha 1H$ protein and exhibited a current density of 53 pA/pF. Three clones (#11, #19, and #25) expressed the rat $\alpha 1I$ protein and exhibited current densities of 40 pA/pF, 45 pA/pF, and 55 pA/pF, respectively.

EXAMPLE 3

This example demonstrates that the cloned putative T-type calcium channels exhibit T-type current-voltage relationships.

Current traces were elicited by depolarizing voltage clamp pulses of the membranes of cells. The $\alpha 1G$, $\alpha 1H$, and $\alpha 1I$ proteins were produced in *Xenopus laevis* oocytes by linearizing the DNA vectors containing the coding sequences, and transcribing the coding sequences *in vitro* by standard methods. Oocytes were then injected with the capped RNA.

Figures 2A-2E depict data obtained from these experiments using cells injected with $\alpha 1G$ (Figure 2A), $\alpha 1H$ (Figure 2B), and $\alpha 1I$ (Figure 2C) and $\alpha 1E$ (Figure 2D). These data indicate that cells expressing $\alpha 1G$, $\alpha 1H$, and $\alpha 1I$ exhibit T-type calcium current, while oocytes expressing $\alpha 1E$ as well as uninjected oocytes (Figure 6A) do not.

Current voltage curves were developed using cells injected with $\alpha 1G$, $\alpha 1H$, $\alpha 1I$, and $\alpha 1E$. Figures 3A depicts such data generated in a 10 mM Ba^{2+} test solution. These data were transformed into conductance and fit with a Boltzman equation to determine the midpoint of activation ($V_{0.5}$). Gating potentials for $\alpha 1G$, $\alpha 1H$, and $\alpha 1I$ (-38 ± 1 mV $n=8$, -44 mV ± 1 mV, $n=10$, and -31 mV ± 1 mV, $n=6$, respectively) were in accordance with the gating potential measured for the HEK-293 cells (-41 ± 1 mV, $n=10$), while $\alpha 1E$ required significantly more positive potentials to open (-2.6 mV $\pm .4$ mV, $n=3$).

To compare the characteristics with published values (Huguenard, *Ann. Rev. Physiol.*, 58, 329-48 (1996)), the $\alpha 1G$ current was recorded at varying concentrations of Ba^{2+} . As indicated in Figure 3B, in solutions containing 2 mM Ba^{2+} , $V_{0.5}$ was -46.5 mV, and the slope factor (k) was 6.6 ($n=7$). However, when the Ba^{2+} concentration was 40 mM, $V_{0.5}$ was recorded at -21 mV, presumably due to the results of barium on surface charge screening (see, e.g., Wilson et al., *J. Membrane Biol.*, 72, 117-30 (1983)). Similar values were recorded for $\alpha 1H$ and $\alpha 1I$.

These results indicate that $\alpha 1G$, $\alpha 1H$, and $\alpha 1I$ are low-voltage activated calcium channels (i.e., from about -60 mV to about -30 mV in 10 mM Ba^{2+}).

EXAMPLE 4

This example demonstrates that the cloned putative T-type calcium channels exhibit T-type tail current.

Tail current was measured at -90 mV after first opening the channels with a voltage step to -10 mV. The voltage-dependence of tail current in cells expressing $\alpha 1G$ (oocytes) $\alpha 1H$ (HEK 293 cells), and $\alpha 1I$ (HEK 293 cells) was measured at varying test potentials. As a control, tail current was also measured from a high voltage activated channel $\alpha 1E$, which Raw data from recordings data were fit with a single exponential and plotted as a function of depolarization potential (Figure 4).

These results demonstrate that the tail currents for the cloned $\alpha 1G$, $\alpha 1H$, and $\alpha 1I$ calcium channels are voltage-dependent, consistent with known T-type calcium tail currents. Additionally, these data demonstrate that the tail current for each of the cloned channels is between about 1 ms and about 10 ms following repolarization to a membrane potential from about -80 mV to about -60 mV in a solution with a barium concentration of from about 10 mM to about 40 mM.

EXAMPLE 5

This example demonstrates that the cloned putative T-type calcium channels exhibit T-type single channel conductance.

Measurement of single channel conductance is complicated by the low probability of channel opening at negative potentials when the driving force is large. Thus, single channel conductance was measured similarly for measurements of tail currents to enhance channel opening at negative potentials. Single channels were measured with standard depolarizing bath and pipette (115 mM $BaCl_2$, 1 mM EGTA, and 10 mM HEPES, pH 7.4) solutions (Lacerda et al., *Biophys. J.*, 66, 1833-43 (1994)). Data were analyzed with TRANSIT (VanDongan, *Biophys. J.*, 70, 1303-15 (1996)). Single channel amplitudes were measured by averaging the values obtained from Gaussian fits to all-points histograms of traces with openings, selected openings, or amplitude histograms of idealized openings. It has been reported that some oocytes contain a native 9 pS channel. These endogenous channels can be distinguished by their 2-fold larger current amplitudes at the potentials tested (e.g., -20 mV, $i = 0.8$ for endogenous channels as opposed to 0.4 pA for $\alpha 1G$). However, such endogenous channels were not detected either at the whole cell or single channel level in the oocytes tested.

Current through the main open state of each open channel was measured at each potential and plotted against each test potential. Single channel currents for several patches were then averaged and plotted as a function of test potential, wherein the slope of the plot indicated the single channel conductance. The average slope conductance of the $\alpha 1G$ channel was measured at 7.5 ± 1.5 pS, which corresponds with the reported values for T-type calcium channels (Hugenard, *Ann. Rev. Physiol.*, 58, 329-48 (1996)). Similar results were also obtained with both $\alpha 1H$ (10.8 ± 1.4 pS). Data collected from recordings of the $\alpha 1I$ channels indicate that they open to two distinct amplitudes. The conductance for the small amplitude $\alpha 1I$ openings was measured at 3.9 ± 0.5 pS, while that for the large $\alpha 1I$ openings was measured at 11.4 ± 0.5 pS).

These results indicate that the cloned $\alpha 1G$, $\alpha 1H$, and $\alpha 1I$ proteins exhibit T-type single-channel conductance (e.g., from about 4 to about 12 pS).

EXAMPLE 6

This example demonstrates that a cloned T-type calcium channel can be used for identifying a drug which affects T-type calcium channels.

5 HEK-293 cells were subjected to treatment as indicated above in Example 3, except that an experimental group of cells were exposed to a solution containing 1 μ M mibefradil, a known inhibitor of T-type calcium current. As depicted in Figure 5A, the presence of mibefradil almost completely abolished T-type current in cells
10 expressing α 1G. Cells expressing either α 1G or α 1H were similarly treated using various concentrations of mibefradil to determine a dose-response relationship. These results, depicted in Figure 5B, demonstrate that about 50% inhibition was achieved at a mibefradil concentration of 1 μ M.

15 All of the references cited herein, including patents, patent applications, and publications, are hereby incorporated in their entireties by reference.

While this invention has been described with an emphasis upon preferred embodiments, it will be obvious to those of ordinary skill in the art that variations of the preferred embodiments may be used and that it is intended that the invention may be practiced otherwise than as specifically described herein. Accordingly, this
20 invention includes all modifications encompassed within the spirit and scope of the invention as defined by the following claims.

What is claimed is:

1. A isolated or substantially purified nucleic acid encoding a protein comprising at least one domain of a T-type calcium channel α subunit.

5 2. The nucleic acid of claim 1, wherein said protein comprises an entire T-type calcium channel α subunit.

3. The nucleic acid of claim 2, wherein said protein comprises SEQ ID NO:13.

10 4. The nucleic acid of any of claims 1-3, wherein said calcium channel begins to gate from about -60 mV to about -30 mV in 2 mM Ba^{2+} .

5. The nucleic acid of any of claims 1-4, wherein said calcium channel exhibits a tail current of from about 1 ms to about 10 ms following repolarization to a membrane potential from about -80 mV to about -60 mV in a solution with a barium concentration of from about 10 mM to about 40 mM.

15 6. The nucleic acid of any of claims 1-5, wherein said calcium channel exhibits a single channel conductance of from about 4 pS to about 11 pS in a solution with a barium ion concentration of about 100 mM.

7. An isolated or substantially purified nucleic acid hybridizing to the nucleic acid of any of claims 1-6.

20 8. An isolated or substantially purified nucleic acid hybridizing to the nucleic acid of claim 7.

9. The nucleic acid of claim 8 comprising a sequence encoding at least one domain of a T-type calcium channel α subunit.

10. A vector comprising the nucleic acid of any of claims 1-9.

25 11. A cell into which the vector of claim 10 has been introduced.

12. The cell of claim 11, which expresses said nucleic acid to produce said protein.

13. The cell of claim 11 or 12, which stably expresses said nucleic acid to produce said protein.

30 14. A population of cells consisting essentially of cells according to any of claims 11-13.

15. An established cell line consisting essentially of cells according to any of claims 11-13.

35 16. A method of identifying a drug which affects T-type calcium channels, said method comprising expressing a T-type calcium channel in a cell, exposing said cell to a putative drug, and measuring the calcium flux through the membrane of said cell in response to a change in membrane potential.

17. The method of claim 16, wherein said calcium flux is assayed by using a calcium-sensitive labile dye within said cell.

18. The method of claim 16, wherein said calcium flux is assayed by measuring the electrophysiological properties of said cell.

5 19. The method of claim 16, wherein said calcium channel comprises SEQ ID NO:13.

20. An isolated or substantially purified immunoglobulin recognizing an epitope on a T-type calcium channel protein.

21. A cell *in vitro* which produces the immunoglobulin of claim 20.

10 22. An established cell line consisting essentially of cells according to claim 21.

hCavT1a MDEEDGAGAEESGQPR-----SFMRLNDLSGAGRPGCPGSAEKDPGSADSEAEGLPYPALAPVVFYLSQDSRPRSWCLRTVCNPNP
rCavT1a MDEEDGAGAEESGQPR-----SFTQLNDLSGAGRPGCPGSTEKDPGSADSEAEGLPYPALAPVVFYLSQDSRPRSWCLRTVCNPNP
hCavT2a MTEGARAADDEVRLGRRPWPCGCGVGGVPEPRCAGTRGGGFFELGVSPSEPAARCAELGADEEQRVPYPALAAATVFFCLGQTRPRSWCLRLVCNPNP
hCavT3 MAESASPPSSSSAAA-----PAAEPGVTTTEQPCPRSPSPSPGLEEPLDGDHPVPHDLPAPIAFFCLRQTTSPRNWCIRKMCNPNP
rCavT3 MADSNLPPSSAAAP-----APEPG--ITEQPCPRSPSPSPGLEEPLDGDHPVPHDLPAPVAFCLRQTTSPRNWCIRKMCNPNP

IS1 IS2 IS3
hCavT1a FERISMLVILLNCVTLMFRPCEDIAQDSQRCRILQAFDDFIFAFAFVEMVVKMVALGIFGKKCYLGDWTNRDLDEFFIVIAAGMLEYSLDLQNVSFSAVRTV
rCavT1a FERVSMVLVILLNCVTLMFRPCEDIAQDSQRCRILQAFDDFIFAFAFVEMVVKMVALGIFGKKCYLGDWTNRDLDEFFIVIAAGMLEYSLDLQNVSFSAVRTV
hCavT2a FEHVSMVLVILLNCVTLMFRPCEDVECGSERCNILEAFDAFIFAFAFVEMVVKMVALGIFGKKCYLGDWTNRDLDEFFIVIAAGMLEYSLDLQNVSLSAIRT
hCavT3 FECVSMVLVILLNCVTLMYQPCDDMDCLSDRCKIMQVDDFIFIFIFAMEMVLKMVALGIFGKKCYLGDWTNRDLDEFFIVIAAGMLEYSLDLQNVINLSAIRT
rCavT3 FECVSMVLVILLNCVTLMYQPCDDMECLSDRCKILQVDDFIFIFIFAMEMVLKMVALGIFGKKCYLGDWTNRDLDEFFIVIAAGMLEYSLDLQNVINLSAIRT

IS4 IS5
hCavT1a RVLRLPLRAINRVPSMRILVTLILLDTLPLMGNVLLLCFFVFFIFGIVGVQLWAGLLNRNRCFLPENFSLPLSVD-LERYYQTENEDESPFICSQPRENGMRS
rCavT1a RVLRLPLRAINRVPSMRILVTLILLDTLPLMGNVLLLCFFVFFIFGIVGVQLWAGLLNRNRCFLPENFSLPLSVD-LEPYQTENEDESPFICSQPRENGMRS
hCavT2a RVLRLPLRAINRVPSMRILVTLILLDTLPLMGNVLLLCFFVFFIFGIVGVQLWAGLLNRNRCFLDSAFVRNNLTFLRPYYQTEEGENPFICSSRRDNGMQK
hCavT3 RVLRLPLKAINRVPSMRILVNLILLDTLPLMGNVLLLCFFVFFIFGIIGVQLWAGLLNRNRCFLEENFTIQGDVA-LPPYYQPEEDDEMPFICSLSGDNGIMG
rCavT3 RVLRLPLKAINRVPSMRILVNLILLDTLPLMGNVLLLCFFVFFIFGIIGVQLWAGLLNRNRCFLEENFTIQGDVA-LPPYYQPEEDDEMPFICSLTGDNGIMG

IP LOOP
hCavT1a CRSVPTLRGDG-----GGPPCGLDYEAAXNSSNTTCVNNQYNTNCSAGEHNPFKGAINFEDNIGYAWIAIFQVITLEGWDMYFVMDAHSFYNYFYFI
rCavT1a CRSVPTLRGEG-----GGPPCSDLYETYNSSNTTCVNNQYNTNCSAGEHNPFKGAINFEDNIGYAWIAIFQVITLEGWDMYFVMDAHSFYNYFYFI
hCavT2a CSHIPGRDVRNPFCTLGWEA-YTQPQAEVGAARNACINWNQYNNVCRSGDSNPHNGAINFEDNTCYAWIAIFQVITLEGWDMYFVMDAHSFYNYFYFI
hCavT3 CHEIPPLKEQGRECCLSKDDVDYDFGAGRODLNASGLCVNNRYNNVCRSGSANPHKGAINFEDNIGYAWIVIFQVITLEGWDMYFVMDAHSFYNYFYFI
rCavT3 CHEIPPLKEQGRECCLSKDDVDYDFGAGRODLNASGLCVNNRYNNVCRSGSANPHKGAINFEDNIGYAWIVIFQVITLEGWDMYFVMDAHSFYNYFYFI

IS6
hCavT1a LLIIVGSFFMINCLVVIATQFSETKQRESQILMREQRVRLSNASTILASFSEPGSCYEEILLKYLIVYLKKAARLAQVSRAAGVVRVGLLSSPAPLGGQET
rCavT1a LLIIVGSFFMINCLVVIATQFSETKQRESQILMREQRVRLSNASTILASFSEPGSCYEEILLKYLIVYLKKAARLAQVSRAIGVRAGLLSSPVARSQEP
hCavT2a LLIIVGSFFMINCLVVIATQFSETKQRESQILMREQRARHLNSDSTILASFSEPGSCYEEILLKYLIVYLKKAARLAQVSRAIGVRAGLLSSPVARSQEP
hCavT3 LLIIVGSFFMINCLVVIATQFSETKQREHRIMLEQRQRYLSS-STVASYAEPGDCYEEIFQYVCHILKKAARLAQVSRAIGVRAGLLSSPVARSQEP
rCavT3 LLIIVGSFFMINCLVVIATQFSETKQREHRIMLEQRQRYLSS-STVASYAEPGDCYEEIFQYVCHILKKAARLAQVSRAIGVRAGLLSSPVARSQEP

Fig. 1A

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hCavT1a QPSSSCSRHRRLSVHHLVHHHHHHHHYHLGNGTLRAPRASPEIQDRDANGSRRLMLPPPSTPALSGAPPGCA-----ESVHSFYHADCHLEPVRC
rCavT1a QPSGCTSRHRRLSVHHLVHHHHHHHHYHLGNGTLRVPRASPEIQDRDANGSRRLMLPPPSTPTSPGPPRGA-----ESVHSFYHADCHLEPVRC
hCavT2a GHRQRAGRHTASVHHLVYHHHHHHHHYHFSHGSPRRPGPEPGACDRLVRACAPPSPSPGCPDAESVHSIYHADCHIEGPQERARVGTCSRSHCRC
hCavT3 -----
rCavT3 -----

hCavT1a QAPPPRSPSEASGRVTGSGKVYPTVHTSPPETLKEKALVEVAASSGPPTLSLN-IPPGPYSSMHKLELTQSTGACQSSCKISSPCLKADSGACGPDSC
rCavT1a QAPPPRCPSEASGRVTGSGKVYPTVHTSPPEILKDKALVEVAPSPGPTLTSFN-IPPGPFSSMHKLELTQSTGACHSSCKISSPCSKADSGACGPDSC
hCavT2a QPQAGHRAGHHELPHDPALRGQRQRQHQPRTQGEVGRWTARHGHGPLSLNSDPYEKIPHVAGEHGLGQAPGHLGSLVPCPLPSPAGTLTCELKSC
hCavT3 -----ALGPEAPAPAKPGPHAKEPRHYQLCPQHSPDLATPHTLVQPIPATLASDPASC
rCavT3 -----AMGPGTPAPAKPGPHAKEPSHCKLCPRHSPLDPTPHTLVQPIAISAILASDPSSC

hCavT1a PYCARA-GAGEVELADREMPDSDSEAVYEFTQDAQHSDLRDPHS-----RR-QRSLGPDAPESPSSVLAFWRLICDTRFKIVDSKYFGRGIM
rCavT1a PYCART-GAGEPESADHVMPDSDSEAVYEFTQDAQHSDLRDPHS-----RRQRSLGPDAPESPSSVLAFWRLICDTRFKIVDSKYFGRGIM
hCavT2a PYCTRALEDPEGELSGSESDSDGRGVYEFTQDVHGRWDPTRPPTATDTPGPGSPQORRAQQAAPCEPGMGRMLVWTFSGKLRRIVDSKYFSRGIM
hCavT3 PCCQHEGRRPGLGSDTSCQEGS-----GSGSSAGCEDEADCGARSEDGASSELGKEEEEEQADGAVLWCGDVWRETRAKLRGIVDSKYFNRGIM
rCavT3 PHCQHEAGRRPGLGSDTSCQEGS-----GSGGSA--EAEANGDGLQSSDGYSDLGKEEQE---DGAARLCGDVWRETRKLRGIVDSKYFNRGIM

IIS1 IIS2 IIS3 IIS4
hCavT1a IAILVNTLSMGIEYHEQPEELTNALEISNIVFTSLFALEMLKLLVYGPFGYIKNPYNI FDGVIIVVISVWEIVGQGGGLSVLRTFLMRVLKLVRFPLPA
rCavT1a IAILVNTLSMGIEYHEQPEELTNALEISNIVFTSLFALEMLKLLVYGPFGYIKNPYNI FDGVIIVVISVWEIVGQGGGLSVLRTFLMRVLKLVRFPLPA
hCavT2a MAILVNTLSMGVEYHEQPEELTNALEISNIVFTSMFALEMLKLLACGPGLYIRNPYNI FDGIIIVVISVWEIVGQADGGLSVLRTFLMRVLKLVRFPLPA
hCavT3 MAILVNTVSMGIEHHEQPEELTNILEICNVVFTSMFALEMILKLAAGFLFDYLRNPYNI FDSIIIVISWEIVGQADGGLSVLRTFLMRVLKLVRFEMPA
rCavT3 MAILVNTVSMGIEHHEQPEELTNILEICNVVFTSMFALEMILKLAAGFLFDYLRNPYNI FDSIIIVISWEIVGQADGGLSVLRTFLMRVLKLVRFEMPA

IIS5 IIP LOOP
hCavT1a LQRQLVLMKTMNVATFCMLLMLEFIFISILGMHLEFGCKFASERD-GDTLPDRKNFDSLMLWAIIVTVFQILTQEDWNKVLNGMASTSSWAALYFIALMT
rCavT1a LQRQLVLMKTMNVATFCMLLMLEFIFISILGMHLEFGCKFASERD-GDTLPDRKNFDSLMLWAIIVTVFQILTQEDWNKVLNGMASTSSWAALYFIALMT
hCavT2a LRRQLVLMKTMNVATFCMLLMLEFIFISILGMHLEFGCKFSLKTDTCGTVDRKNFDSLMLWAIIVTVFQILTQEDWNVVLNGMASTSSWAALYFVALMT
hCavT3 LRRQLVLMKTMNVATFCMLLMLEFIFISILGMHIFGCKFSLRDTGTCGTVDRKNFDSLMLWAIIVTVFQILTQEDWNVVLNGMASTSPWASLYFVALMT
rCavT3 LRRQLVLMKTMNVATFCMLLMLEFIFISILGMHIFGCKFSLRDTGTCGTVDRKNFDSLMLWAIIVTVFQILTQEDWNVVLNGMASTTPWASLYFVALMT

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Fig. 1B

IIIS6

hCavT1a FGNVLFNLLVAAILVEGFOAEGDANKSESEPDFFSPSLDGDGRKKCLALVSLGEHPELRKSLPLP-----IIHTAATPMSLPKSTSTGLGEALGPASR
rCavT1a FGNVLFNLLVAAILVEGFOAEGDANKSESEPDFFSPSLDGDGRKKRLALVALGEHAEIRKSLPLP-----IIHTAATPMSHPKSSSTGVGEALGSGSR
hCavT2a FGNVLFNLLVAAILVEGFOAEGDANKSDTDEDKTSVHEEDFKLRELQTTLMKSLAVTPNGTWRDEAACLPSPSSCAQLPRPCLPPRAHHSWMQPPAS
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Fig. 1C

Fig. 1D

```

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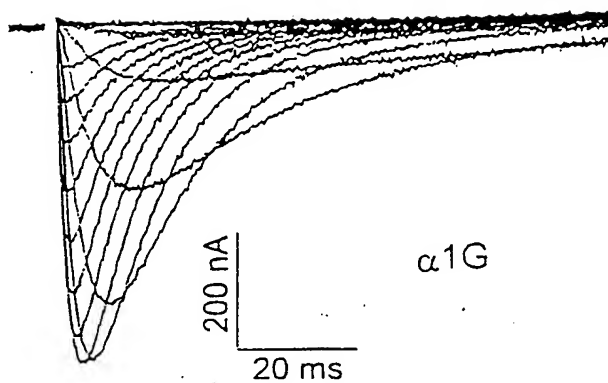
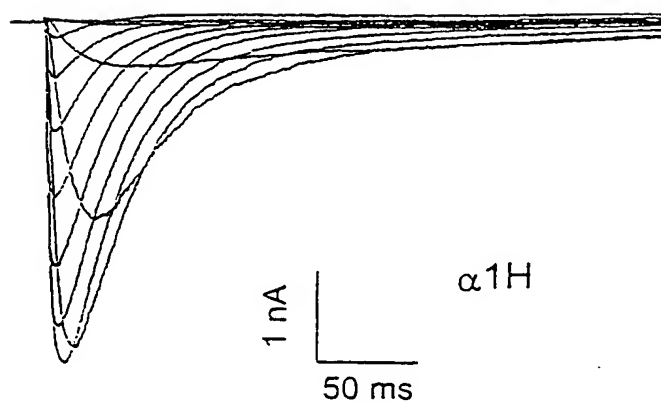
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rCavT3  -----LEGELTIIDNLGSIHFHYASPDGCGKCHHDKQETGLHPSCWGMT (SEQ ID NO:12)

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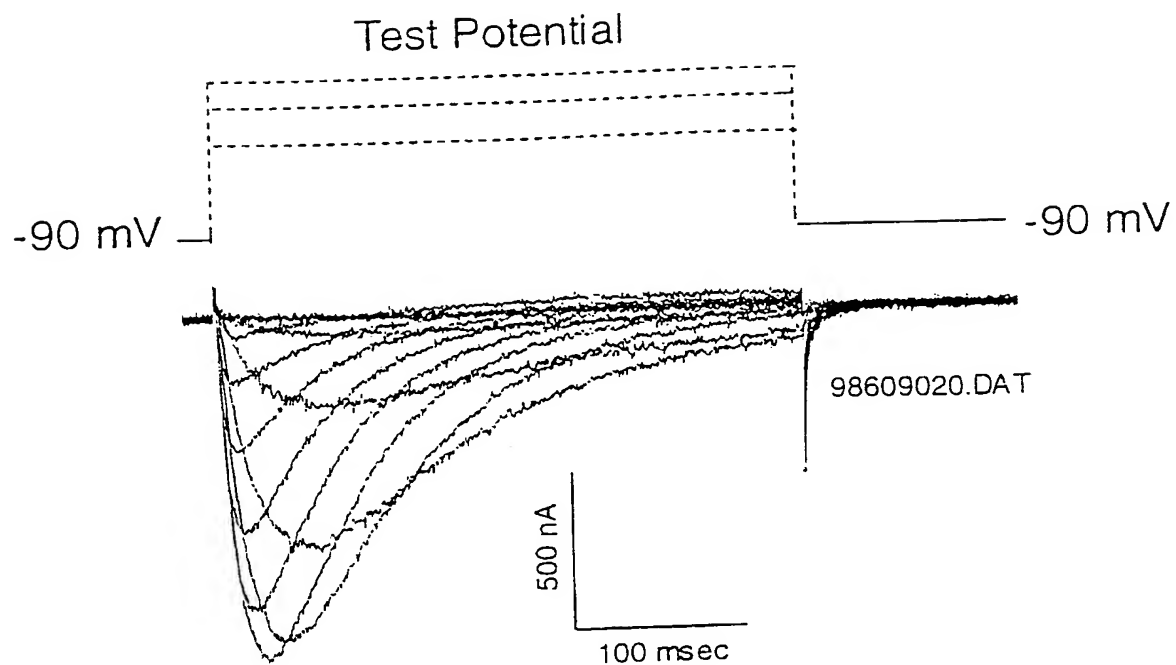
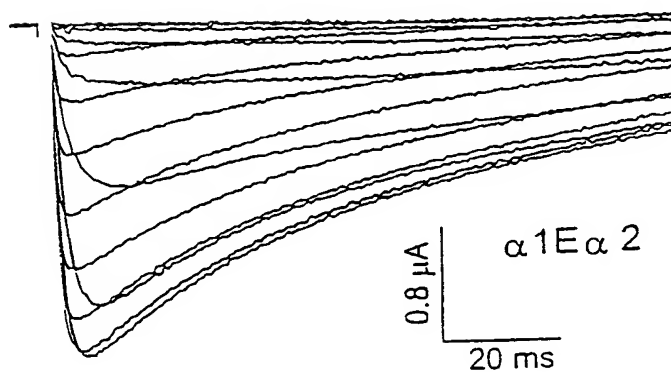
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rCavT1a STAASPSPKKDTLSLSGLSSDPTDMDP (SEQ ID NO:5)

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Fig. 1E

**Figure 2A****Figure 2B**

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**Figure 2C****Figure 2D**

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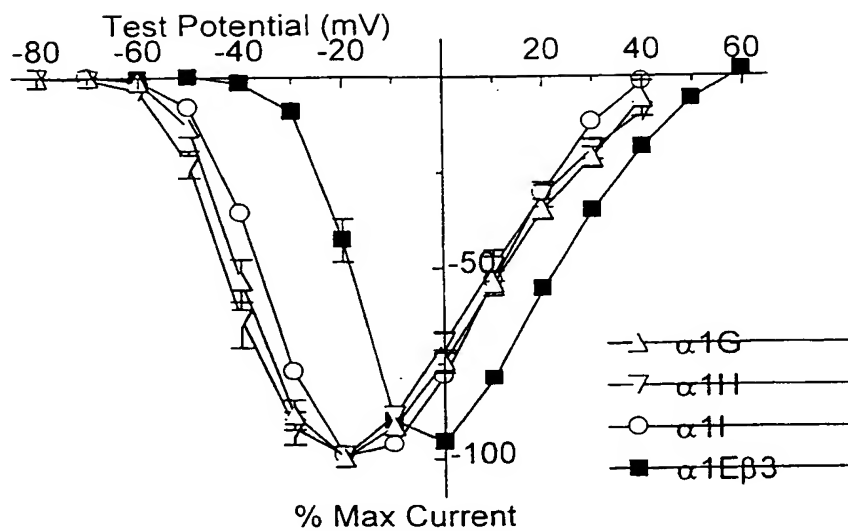


Figure 3A

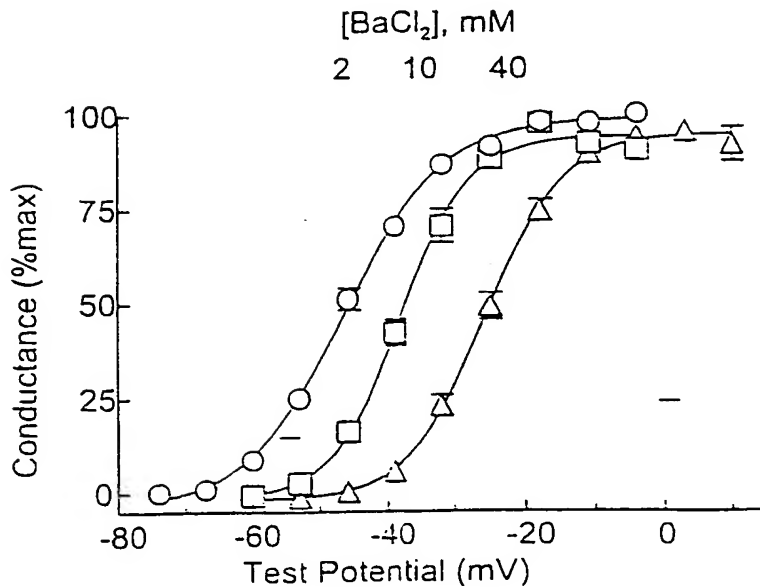
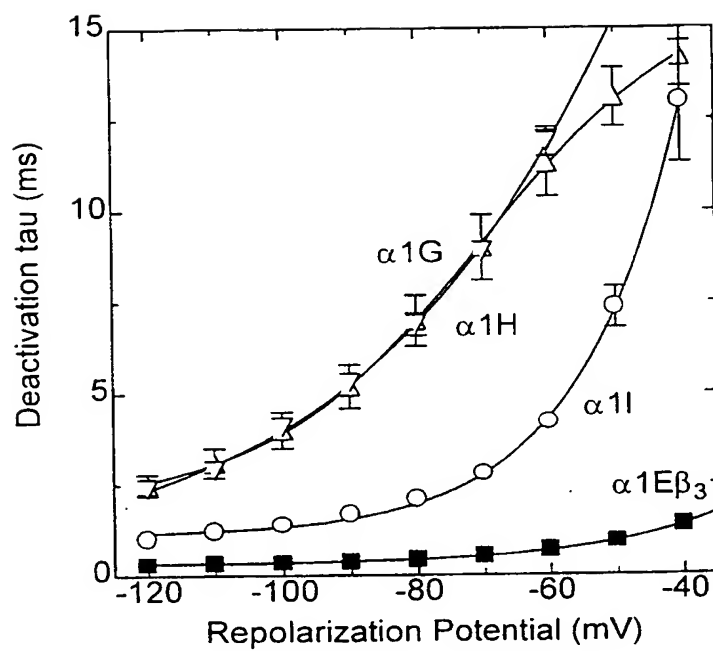
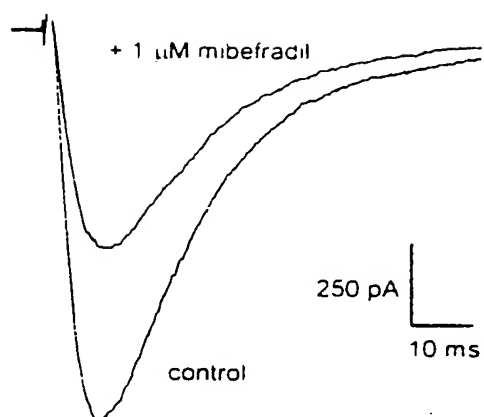
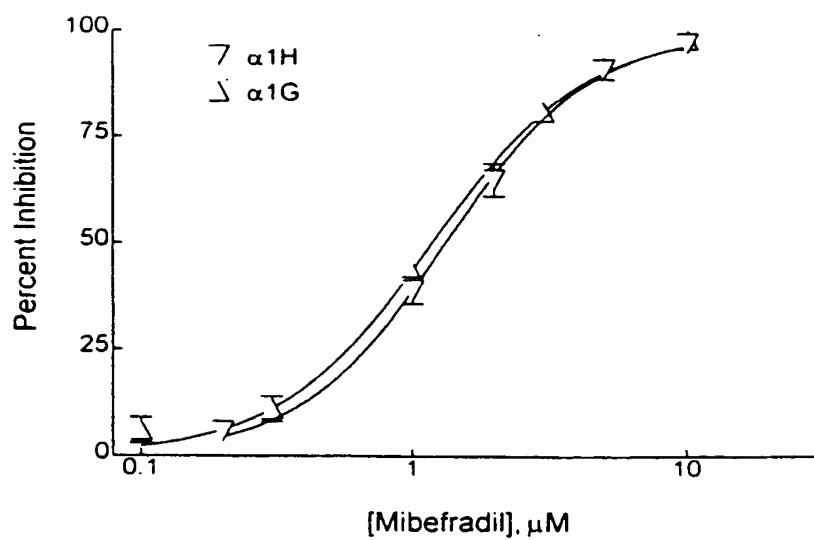


Figure 3B

**Figure 4**

**Figure 5A****Figure 5B**

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 Cribbs, Leanne L.
 5 Loyola University of Chicago

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35	gag	gag	ctt	acc	aac	gcc	cta	gaa	atc	agc	aac	atc	gtc	ttc	acc	agc	2352
	Glu	Glu	Leu	Thr	Asn	Ala	Leu	Glu	Ile	Ser	Asn	Ile	Val	Phe	Thr	Ser	
			770				775					780					
	ctc	ttt	gcc	ctg	gag	atg	ctg	ctg	aag	ctg	ctt	gtg	tat	ggt	ccc	ttt	2400
	Leu	Phe	Ala	Leu	Glu	Met	Leu	Leu	Lys	Leu	Leu	Val	Tyr	Gly	Pro	Phe	
	785					790				795						800	
40	ggc	tac	atc	aag	aat	ccc	tac	aac	atc	ttc	gat	ggt	gtc	att	gtg	gtc	2448
	Gly	Tyr	Ile	Lys	Asn	Pro	Tyr	Asn	Ile	Phe	Asp	Gly	Val	Ile	Val	Val	
					805					810					815		
45	atc	agc	gtg	tgg	gag	atc	gtg	ggc	cag	cag	ggg	ggc	ggc	ctg	tcg	gtg	2496
	Ile	Ser	Val	Trp	Glu	Ile	Val	Gly	Gln	Gln	Gly	Gly	Gly	Leu	Ser	Val	
				820					825					830			
50	ctg	cgg	acc	ttc	cgc	ctg	atg	cgt	gtg	ctg	aag	ctg	gtg	cgc	ttc	ctg	2544
	Leu	Arg	Thr	Phe	Arg	Leu	Met	Arg	Val	Leu	Lys	Leu	Val	Arg	Phe	Leu	
				835				840					845				
55	ccg	gcg	ctg	cag	cgg	cag	ctg	gtg	gtg	ctc	atg	aag	acc	atg	gac	aac	2592
	Pro	Ala	Leu	Gln	Arg	Gln	Leu	Val	Val	Leu	Met	Lys	Thr	Met	Asp	Asn	
				850			855					860					
	gtg	gcc	acc	ttc	tgc	atg	ctg	ctt	atg	ctc	ttc	atc	ttc	atc	ttc	agc	2640
	Val	Ala	Thr	Phe	Cys	Met	Leu	Leu	Met	Leu	Phe	Ile	Phe	Ile	Phe	Ser	
						870					875					880	
60	atc	ctg	ggc	atg	cat	ctc	ttc	ggc	tgc	aag	ttt	gcc	tct	gag	cgg	gat	2688
	Ile	Leu	Gly	Met	His	Leu	Phe	Gly	Cys	Lys	Phe	Ala	Ser	Glu	Arg	Asp	
					885					890					895		
	ggg	gac	acc	ctg	cca	gac	cgg	aag	aat	ttt	gac	tcc	ttg	ctc	tgg	gcc	2736

	Gly	Asp	Thr	Leu	Pro	Asp	Arg	Lys	Asn	Phe	Asp	Ser	Leu	Leu	Trp	Ala	
				900					905					910			
5	atc	gtc	act	gtc	ttt	cag	atc	ctg	acc	cag	gag	gac	tgg	aac	aaa	gtc	2784
	Ile	Val	Thr	Val	Phe	Gln	Ile	Leu	Thr	Gln	Glu	Asp	Trp	Asn	Lys	Val	
			915					920					925				
10	ctc	tac	aat	ggg	atg	gcc	tcc	acg	tcg	tcc	tgg	gcg	gcc	ctt	tat	ttc	2832
	Leu	Tyr	Asn	Gly	Met	Ala	Ser	Thr	Ser	Ser	Trp	Ala	Ala	Leu	Tyr	Phe	
			930				935					940					
15	att	gcc	ctc	atg	acc	ttc	ggc	aac	tac	gtg	ctc	ttc	aat	ttg	ctg	gtc	2880
	Ile	Ala	Leu	Met	Thr	Phe	Gly	Asn	Tyr	Val	Leu	Phe	Asn	Leu	Leu	Val	
	945					950					955					960	
	gcc	att	ctg	gtg	gag	ggc	ttc	cag	gcg	gag	gga	gat	gcc	aac	aag	tcc	2928
	Ala	Ile	Leu	Val	Glu	Gly	Phe	Gln	Ala	Glu	Gly	Asp	Ala	Asn	Lys	Ser	
					965					970					975		
20	gaa	tca	gag	ccc	gat	ttc	ttc	tca	ccc	agc	ctg	gat	ggg	gat	ggg	gac	2976
	Glu	Ser	Glu	Pro	Asp	Phe	Phe	Ser	Pro	Ser	Leu	Asp	Gly	Asp	Gly	Asp	
				980					985					990			
25	agg	aag	aag	tgc	ttg	gcc	ttg	gtg	tcc	ctg	gga	gag	cac	ccg	gag	ctg	3024
	Arg	Lys	Lys	Cys	Leu	Ala	Leu	Val	Ser	Leu	Gly	Glu	His	Pro	Glu	Leu	
			995					1000					1005				
30	cgg	aag	agc	ctg	ctg	ccg	cct	ctc	atc	atc	cac	acg	gcc	gcc	aca	ccc	3072
	Arg	Lys	Ser	Leu	Leu	Pro	Pro	Leu	Ile	Ile	His	Thr	Ala	Ala	Thr	Pro	
		1010					1015					1020					
35	atg	tcg	ctg	ccc	aag	agc	acc	agc	acg	ggc	ctg	ggc	gag	gcg	ctg	ggc	3120
	Met	Ser	Leu	Pro	Lys	Ser	Thr	Ser	Thr	Gly	Leu	Gly	Glu	Ala	Leu	Gly	
	1025					1030					1035					1040	
	cct	gcg	tcg	cgc	cgc	acc	agc	agc	agc	ggg	tcg	gca	gag	cct	ggg	gcg	3168
	Pro	Ala	Ser	Arg	Arg	Thr	Ser	Ser	Ser	Gly	Ser	Ala	Glu	Pro	Gly	Ala	
					1045					1050					1055		
40	gcc	cac	gag	atg	aag	tca	ccg	ccc	agc	gcc	cgc	agc	tct	ccg	cac	agc	3216
	Ala	His	Glu	Met	Lys	Ser	Pro	Pro	Ser	Ala	Arg	Ser	Ser	Pro	His	Ser	
				1060					1065					1070			
45	ccc	tgg	agc	gct	gca	agc	agc	tgg	acc	agc	agg	cgc	tcc	agc	cgg	aac	3264
	Pro	Trp	Ser	Ala	Ala	Ser	Ser	Trp	Thr	Ser	Arg	Arg	Ser	Ser	Arg	Asn	
			1075					1080					1085				
50	agc	ctc	ggc	cgt	gca	ccc	agc	ctg	aag	cgg	aga	agc	cca	agt	gga	gag	3312
	Ser	Leu	Gly	Arg	Ala	Pro	Ser	Leu	Lys	Arg	Arg	Ser	Pro	Ser	Gly	Glu	
		1090					1095					1100					
55	cgg	cgg	tcc	ctg	ttg	tcg	gga	gaa	ggc	cag	gag	agc	cag	gat	gaa	gag	3360
	Arg	Arg	Ser	Leu	Leu	Ser	Gly	Glu	Gly	Gln	Glu	Ser	Gln	Asp	Glu	Glu	
	1105					1110					1115					1120	
	gag	agc	tca	gaa	gag	gag	cgg	gcc	agc	cct	gcg	ggc	agt	gac	cat	cgc	3408
	Glu	Ser	Ser	Glu	Glu	Glu	Arg	Ala	Ser	Pro	Ala	Gly	Ser	Asp	His	Arg	
					1125					1130					1135		
60	cac	agg	ggg	tcc	ctg	gag	cgg	gag	gcc	aag	agt	tcc	ttt	gac	ctg	cca	3456
	His	Arg	Gly	Ser	Leu	Glu	Arg	Glu	Ala	Lys	Ser	Ser	Phe	Asp	Leu	Pro	
				1140					1145					1150			
	gac	aca	ctg	cag	gtg	cca	ggg	ctg	cat	cgc	act	gcc	agt	ggc	cga	ggg	3504

	Asp Thr Leu Gln Val Pro Gly Leu His Arg Thr Ala Ser Gly Arg Gly	
	1155 1160 1165	
5	tct gct tct gag cac cag gac tgc aat ggc aag tcg gct tca ggg cgc Ser Ala Ser Glu His Gln Asp Cys Asn Gly Lys Ser Ala Ser Gly Arg	3552
	1170 1175 1180	
10	ctg gcc cgg gcc ctg cgg cct gat gac ccc cca ctg gat ggg gat gac Leu Ala Arg Ala Leu Arg Pro Asp Asp Pro Pro Leu Asp Gly Asp Asp	3600
	1185 1190 1195 1200	
15	gcc gat gac gag ggc aac ctg agc aaa ggg gaa cgg gtc cgc gcg tgg Ala Asp Asp Glu Gly Asn Leu Ser Lys Gly Glu Arg Val Arg Ala Trp	3648
	1205 1210 1215	
	atc cga gcc cga ctc cct gcc tgc tgc ctc gag cga gac tcc tgg tca Ile Arg Ala Arg Leu Pro Ala Cys Cys Leu Glu Arg Asp Ser Trp Ser	3696
	1220 1225 1230	
20	gcc tac atc ttc cct cct cag tcc agg ttc cgc ctc ctg tgt cac cgg Ala Tyr Ile Phe Pro Pro Gln Ser Arg Phe Arg Leu Leu Cys His Arg	3744
	1235 1240 1245	
25	atc atc acc cac aag atg ttc gac cac gtg gtc ctt gtc atc atc ttc Ile Ile Thr His Lys Met Phe Asp His Val Val Leu Val Ile Ile Phe	3792
	1250 1255 1260	
30	ctt aac tgc atc acc atc gcc atg gag cgc ccc aaa att gac ccc cac Leu Asn Cys Ile Thr Ile Ala Met Glu Arg Pro Lys Ile Asp Pro His	3840
	1265 1270 1275 1280	
35	agc gct gaa cgc atc ttc ctg acc ctc tcc aat tac atc ttc acc gca Ser Ala Glu Arg Ile Phe Leu Thr Leu Ser Asn Tyr Ile Phe Thr Ala	3888
	1285 1290 1295	
	gtc ttt ctg gct gaa atg aca gtg aag gtg gtg gca ctg ggc tgg tgc Val Phe Leu Ala Glu Met Thr Val Lys Val Val Ala Leu Gly Trp Cys	3936
	1300 1305 1310	
40	ttc ggg gag cag gcg tac ctg cgg agc agt tgg aac gtg ctg gac ggg Phe Gly Glu Gln Ala Tyr Leu Arg Ser Ser Trp Asn Val Leu Asp Gly	3984
	1315 1320 1325	
45	ctg ttg gtg ctc atc tcc gtc atc gac att ctg gtg tcc atg gtc tct Leu Leu Val Leu Ile Ser Val Ile Asp Ile Leu Val Ser Met Val Ser	4032
	1330 1335 1340	
50	gac agc ggc acc aag atc ctg ggc atg ctg agg gtg ctg cgg ctg ctg Asp Ser Gly Thr Lys Ile Leu Gly Met Leu Arg Val Leu Arg Leu Leu	4080
	1345 1350 1355 1360	
55	cgg acc ctg cgc ccg ctc agg gtg atc agc cgg gcg cag ggg ctg aag Arg Thr Leu Arg Pro Leu Arg Val Ile Ser Arg Ala Gln Gly Leu Lys	4128
	1365 1370 1375	
	ctg gtg gtg gag acg ctg atg tcc tca ctg aaa ccc atc ggc aac att Leu Val Val Glu Thr Leu Met Ser Ser Leu Lys Pro Ile Gly Asn Ile	4176
	1380 1385 1390	
60	gta gtc atc tgc tgt gcc ttc ttc atc att ttc ggc atc ttg ggg gtg Val Val Ile Cys Cys Ala Phe Phe Ile Ile Phe Gly Ile Leu Gly Val	4224
	1395 1400 1405	
	cag ctc ttc aaa ggg aag ttt ttc gtg tgc cag ggc gag gat acc agg	4272

	Gln	Leu	Phe	Lys	Gly	Lys	Phe	Phe	Val	Cys	Gln	Gly	Glu	Asp	Thr	Arg	
	1410						1415					1420					
5	aac	atc	acc	aat	aaa	tcg	gac	tgt	gcc	gag	gcc	agt	tac	cgg	tgg	gtc	4320
	Asn	Ile	Thr	Asn	Lys	Ser	Asp	Cys	Ala	Glu	Ala	Ser	Tyr	Arg	Trp	Val	
	1425					1430				1435					1440		
10	cgg	cac	aag	tac	aac	ttt	gac	aac	ctt	ggc	cag	gcc	ctg	atg	tcc	ctg	4368
	Arg	His	Lys	Tyr	Asn	Phe	Asp	Asn	Leu	Gly	Gln	Ala	Leu	Met	Ser	Leu	
					1445					1450					1455		
15	ttc	gtt	ttg	gcc	tcc	aag	gat	ggt	tgg	gtg	gac	atc	atg	tac	gat	ggg	4416
	Phe	Val	Leu	Ala	Ser	Lys	Asp	Gly	Trp	Val	Asp	Ile	Met	Tyr	Asp	Gly	
				1460					1465					1470			
20	ctg	gat	gct	gtg	ggc	gtg	gac	cag	cag	ccc	atc	atg	aac	cac	aac	ccc	4464
	Leu	Asp	Ala	Val	Gly	Val	Asp	Gln	Gln	Pro	Ile	Met	Asn	His	Asn	Pro	
		1475					1480					1485					
25	tgg	atg	ctg	ctg	tac	ttc	atc	tcg	ttc	ctg	ctc	att	gtg	gcc	ttc	ttt	4512
	Trp	Met	Leu	Leu	Tyr	Phe	Ile	Ser	Phe	Leu	Leu	Ile	Val	Ala	Phe	Phe	
	1490					1495						1500					
30	gtc	ctg	aac	atg	ttt	gtg	ggt	gtg	gtg	gtg	gag	aac	ttc	cac	aag	tgt	4560
	Val	Leu	Asn	Met	Phe	Val	Gly	Val	Val	Val	Glu	Asn	Phe	His	Lys	Cys	
	1505				1510					1515					1520		
35	cgg	cag	cac	cag	gag	gaa	gag	gag	gcc	cgg	cgg	cgg	gag	gag	aag	cgc	4608
	Arg	Gln	His	Gln	Glu	Glu	Glu	Glu	Ala	Arg	Arg	Arg	Glu	Glu	Lys	Arg	
				1525					1530						1535		
40	cta	cga	aga	ctg	gag	aaa	aag	aga	agg	agt	aag	gag	aag	cag	atg	gct	4656
	Leu	Arg	Arg	Leu	Glu	Lys	Lys	Arg	Arg	Ser	Lys	Glu	Lys	Gln	Met	Ala	
				1540				1545						1550			
45	gaa	gcc	cag	tgc	aaa	cct	tac	tac	tcc	gac	tac	tcc	cgc	ttc	cgg	ctc	4704
	Glu	Ala	Gln	Cys	Lys	Pro	Tyr	Tyr	Ser	Asp	Tyr	Ser	Arg	Phe	Arg	Leu	
	1555					1560						1565					
50	ctc	gtc	cac	cac	ttg	tgc	acc	agc	cac	tac	ctg	gac	ctc	ttc	atc	aca	4752
	Leu	Val	His	His	Leu	Cys	Thr	Ser	His	Tyr	Leu	Asp	Leu	Phe	Ile	Thr	
	1570				1575						1580						
55	ggt	gtc	atc	ggg	ctg	aac	gtg	gtc	acc	atg	gcc	atg	gag	cac	tac	cag	4800
	Gly	Val	Ile	Gly	Leu	Asn	Val	Val	Thr	Met	Ala	Met	Glu	His	Tyr	Gln	
	1585				1590				1595						1600		
60	cag	ccc	cag	att	ctg	gat	gag	gct	ctg	aag	atc	tgc	aac	tac	atc	ttc	4848
	Gln	Pro	Gln	Ile	Leu	Asp	Glu	Ala	Leu	Lys	Ile	Cys	Asn	Tyr	Ile	Phe	
				1605				1610						1615			
65	act	gtc	atc	ttt	gtc	ttg	gag	tca	gtt	ttc	aaa	ctt	gtg	gcc	ttt	ggt	4896
	Thr	Val	Ile	Phe	Val	Leu	Glu	Ser	Val	Phe	Lys	Leu	Val	Ala	Phe	Gly	
				1620				1625					1630				
70	ttc	cgt	cgg	ttc	ttc	cag	gac	agg	tgg	aac	cag	ctg	gac	ctg	gcc	att	4944
	Phe	Arg	Arg	Phe	Phe	Gln	Asp	Arg	Trp	Asn	Gln	Leu	Asp	Leu	Ala	Ile	
		1635					1640					1645					
75	gtg	ctg	ctg	tcc	atc	atg	ggc	atc	acg	ctg	gag	gaa	atc	gag	gtc	aac	4992
	Val	Leu	Leu	Ser	Ile	Met	Gly	Ile	Thr	Leu	Glu	Glu	Ile	Glu	Val	Asn	
	1650					1655					1660						
80	gcc	tcg	ctg	ccc	atc	aac	ccc	acc	atc	atc	cgc	atc	atg	agg	gtg	ctg	5040

	Ala Ser Leu Pro Ile Asn Pro Thr Ile Ile Arg Ile Met Arg Val Leu	
	1665 1670 1675 1680	
5	cgc att gcc cga gtg ctg aag ctg ctg aag atg gct gtg ggc atg cgg Arg Ile Ala Arg Val Leu Lys Leu Leu Lys Met Ala Val Gly Met Arg	5088
	1685 1690 1695	
10	gcg ctg ctg gac acg gtg atg cag gcc ctg ccc cag gtg ggg aac ctg Ala Leu Leu Asp Thr Val Met Gln Ala Leu Pro Gln Val Gly Asn Leu	5136
	1700 1705 1710	
15	gga ctt ctc ttc atg ttg ttg ttt ttc atc ttt gca gct ctg ggc gtg Gly Leu Leu Phe Met Leu Leu Phe Phe Ile Phe Ala Ala Leu Gly Val	5184
	1715 1720 1725	
	gag ctc ttt gga gac ctg gag tgt gac gag aca cac ccc tgt gag ggc Glu Leu Phe Gly Asp Leu Glu Cys Asp Glu Thr His Pro Cys Glu Gly	5232
	1730 1735 1740	
20	ctg ggc cgt cat gcc acc ttt cgg aac ttt ggc atg gcc ttc cta acc Leu Gly Arg His Ala Thr Phe Arg Asn Phe Gly Met Ala Phe Leu Thr	5280
	1745 1750 1755 1760	
25	ctc ttc cga gtc tcc aca ggt gac aat tgg aat ggc att atg aag gac Leu Phe Arg Val Ser Thr Gly Asp Asn Trp Asn Gly Ile Met Lys Asp	5328
	1765 1770 1775	
30	acc ctc cgg gac tgt gac cag gag tcc acc tgc tac aac acg gtc atc Thr Leu Arg Asp Cys Asp Gln Glu Ser Thr Cys Tyr Asn Thr Val Ile	5376
	1780 1785 1790	
35	tcg cct atc tac ttt gtg tcc ttc gtg ctg acg gcc cag ttc gtg cta Ser Pro Ile Tyr Phe Val Ser Phe Val Leu Thr Ala Gln Phe Val Leu	5424
	1795 1800 1805	
	gtc aac gtg gtg atc gcc gtg ctg atg aag cac ctg gag gag agc aac Val Asn Val Val Ile Ala Val Leu Met Lys His Leu Glu Glu Ser Asn	5472
	1810 1815 1820	
40	aag gag gcc aag gag gag gcc gag cta gag gct gag ctg gag ctg gag Lys Glu Ala Lys Glu Glu Ala Glu Leu Glu Ala Glu Leu Glu Leu Glu	5520
	1825 1830 1835 1840	
45	atg aag acc ctc agc ccc cag ccc cac tcg cca ctg ggc agc ccc ttc Met Lys Thr Leu Ser Pro Gln Pro His Ser Pro Leu Gly Ser Pro Phe	5568
	1845 1850 1855	
50	ctc tgg cct ggg gtc gag ggc ccc gac agc ccc gac agc ccc aag cct Leu Trp Pro Gly Val Glu Gly Pro Asp Ser Pro Asp Ser Pro Lys Pro	5616
	1860 1865 1870	
55	ggg gct ctg cac cca gcg gcc cac gcg aga tca gcc tcc cac ttt tcc Gly Ala Leu His Pro Ala Ala His Ala Arg Ser Ala Ser His Phe Ser	5664
	1875 1880 1885	
	ctg gag cac ccc acg atg cag ccc cac ccc acg gag ctg cca gga cca Leu Glu His Pro Thr Met Gln Pro His Pro Thr Glu Leu Pro Gly Pro	5712
	1890 1895 1900	
60	gac tta ctg act gtg cgg aag tct ggg gtc agc cga acg cac tct ctg Asp Leu Leu Thr Val Arg Lys Ser Gly Val Ser Arg Thr His Ser Leu	5760
	1905 1910 1915 1920	
	ccc aat gac agc tac atg tgt cgg cat ggg agc act gcc gag ggg ccc	5808

	Pro	Asn	Asp	Ser	Tyr	Met	Cys	Arg	His	Gly	Ser	Thr	Ala	Glu	Gly	Pro	
				1925						1930					1935		
5	ctg	gga	cac	agg	ggc	tgg	ggg	ctc	ccc	aaa	gct	cag	tca	ggc	tcc	gtc	5556
	Leu	Gly	His	Arg	Gly	Trp	Gly	Leu	Pro	Lys	Ala	Gln	Ser	Gly	Ser	Val	
			1940					1945					1950				
10	ttg	tcc	gtt	cac	tcc	cag	cca	gca	gat	acc	agc	tac	atc	ctg	cag	ctt	5904
	Leu	Ser	Val	His	Ser	Gln	Pro	Ala	Asp	Thr	Ser	Tyr	Ile	Leu	Gln	Leu	
			1955					1960					1965				
15	ccc	aaa	gat	gca	cct	cat	ctg	ctc	cag	ccc	cac	agc	gcc	cca	acc	tgg	5952
	Pro	Lys	Asp	Ala	Pro	His	Leu	Leu	Gln	Pro	His	Ser	Ala	Pro	Thr	Trp	
		1970					1975					1980					
20	ggc	acc	atc	ccc	aaa	ctg	ccc	cca	cca	gga	cgc	tcc	cct	ttg	gct	cag	6000
	Gly	Thr	Ile	Pro	Lys	Leu	Pro	Pro	Pro	Gly	Arg	Ser	Pro	Leu	Ala	Gln	
	1985					1990					1995					2000	
25	agg	cca	ctc	agg	cgc	cag	gca	gca	ata	agg	act	gac	tcc	ttg	gac	gtt	6048
	Arg	Pro	Leu	Arg	Arg	Gln	Ala	Ala	Ile	Arg	Thr	Asp	Ser	Leu	Asp	Val	
				2005						2010					2015		
30	cag	ggt	ctg	ggc	agc	cgg	gaa	gac	ctg	ctg	gca	gag	gtg	agt	ggg	ccc	6096
	Gln	Gly	Leu	Gly	Ser	Arg	Glu	Asp	Leu	Leu	Ala	Glu	Val	Ser	Gly	Pro	
			2020					2025					2030				
35	tcc	ccg	ccc	ctg	gcc	cgg	gcc	tac	tct	ttc	tgg	ggc	cag	tca	agt	acc	6144
	Ser	Pro	Pro	Leu	Ala	Arg	Ala	Tyr	Ser	Phe	Trp	Gly	Gln	Ser	Ser	Thr	
		2035					2040						2045				
40	cag	gca	cag	cag	cac	tcc	cgc	agc	cac	agc	aag	atc	tcc	aag	cac	atg	6192
	Gln	Ala	Gln	Gln	His	Ser	Arg	Ser	His	Ser	Lys	Ile	Ser	Lys	His	Met	
		2050					2055					2060					
45	acc	ccg	cca	gcc	cct	tgc	cca	ggc	cca	gaa	ccc	aac	tgg	ggc	aag	ggc	6240
	Thr	Pro	Pro	Ala	Pro	Cys	Pro	Gly	Pro	Glu	Pro	Asn	Trp	Gly	Lys	Gly	
	2065				2070						2075					2080	
50	cct	cca	gag	acc	aga	agc	agc	tta	gag	ttg	gac	acg	gag	ctg	agc	tgg	6288
	Pro	Pro	Glu	Thr	Arg	Ser	Ser	Leu	Glu	Leu	Asp	Thr	Glu	Leu	Ser	Trp	
				2085					2090						2095		
55	att	tca	gga	gac	ctc	ctg	ccc	cct	ggc	ggc	cag	gag	gag	ccc	cca	tcc	6336
	Ile	Ser	Gly	Asp	Leu	Leu	Pro	Pro	Gly	Gly	Gln	Glu	Glu	Pro	Pro	Ser	
			2100						2105				2110				
60	cca	cgg	gac	ctg	aag	aag	tgc	tac	agc	gtg	gag	gcc	cag	agc	tgc	cag	6384
	Pro	Arg	Asp	Leu	Lys	Lys	Cys	Tyr	Ser	Val	Glu	Ala	Gln	Ser	Cys	Gln	
		2115						2120				2125					
65	cgc	cgg	cct	acg	tcc	tgg	ctg	gat	gag	cag	agg	aga	cac	tct	atc	gcc	6432
	Arg	Arg	Pro	Thr	Ser	Trp	Leu	Asp	Glu	Gln	Arg	Arg	His	Ser	Ile	Ala	
		2130					2135					2140					
70	gtc	agc	tgc	ctg	gac	agc	ggc	tcc	caa	ccc	cac	ctg	ggc	aca	gac	ccc	6480
	Val	Ser	Cys	Leu	Asp	Ser	Gly	Ser	Gln	Pro	His	Leu	Gly	Thr	Asp	Pro	
	2145					2150					2155					2160	
75	tct	aac	ctt	ggg	ggc	cag	cct	ctt	ggg	ggg	cct	ggg	agc	cgg	ccc	aag	6528
	Ser	Asn	Leu	Gly	Gly	Gln	Pro	Leu	Gly	Gly	Pro	Gly	Ser	Arg	Pro	Lys	
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Lys Lys Leu Ser Pro Pro Ser Ile Thr Ile Asp Pro Pro Glu Ser Gln
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 Gly Pro Arg Thr Pro Pro Ser Pro Gly Ile Cys Leu Arg Arg Arg Ala
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 Arg Ser Phe Met Arg Leu Asn Asp Leu Ser Gly Ala Gly Gly Arg Pro
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 Gly Pro Gly Ser Ala Glu Lys Asp Pro Gly Ser Ala Asp Ser Glu Ala
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 Glu Gly Leu Pro Tyr Pro Ala Leu Ala Pro Val Val Phe Phe Tyr Leu
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 Ser Gln Asp Ser Arg Pro Arg Ser Trp Cys Leu Arg Thr Val Cys Asn
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 Pro Trp Phe Glu Arg Ile Ser Met Leu Val Ile Leu Leu Asn Cys Val
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 Thr Leu Gly Met Phe Arg Pro Cys Glu Asp Ile Ala Cys Asp Ser Gln
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 Arg Cys Arg Ile Leu Gln Ala Phe Asp Asp Phe Ile Phe Ala Phe Phe
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gcc gtg gag atg gtg gtg aag atg gtg gcc ttg ggc atc ttt ggg aaa 432
 Ala Val Glu Met Val Val Lys Met Val Ala Leu Gly Ile Phe Gly Lys

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	His His His His His His His His Tyr His Leu Gly Asn Gly Thr Leu							
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	Glu Val Ala Ala Ser Ser Gly Pro Pro Thr Leu Thr Ser Leu Asn Ile							
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75	cag gac agg tgg aac cag ctg gac ctg gcc att gtg ctg ctg tcc atc Gln Asp Arg Trp Asn Gln Leu Asp Leu Ala Ile Val Leu Leu Ser Ile 1650 1655 1660	4992		
80	atg ggc atc acg ctg gag gaa atc gag gtc aac gcc tcg ctg ccc atc Met Gly Ile Thr Leu Glu Glu Ile Glu Val Asn Ala Ser Leu Pro Ile	5040		

	1665	1670	1675	1680	
5	aac ccc acc atc atc cgc atc atg agg gtg ctg cgc att gcc cga gtg Asn Pro Thr Ile Ile Arg Ile Met Arg Val Leu Arg Ile Ala Arg Val	1685	1690	1695	5088
10	ctg aag ctg ctg aag atg gct gtg ggc atg cgg gcg ctg ctg gac acg Leu Lys Leu Leu Lys Met Ala Val Gly Met Arg Ala Leu Leu Asp Thr	1700	1705	1710	5136
15	gtg atg cag gcc ctg ccc cag gtg ggg aac ctg gga ctt ctc ttc atg Val Met Gln Ala Leu Pro Gln Val Gly Asn Leu Gly Leu Leu Phe Met	1715	1720	1725	5184
20	ttg ttg ttt ttc atc ttt gca gct ctg ggc gtg gag ctc ttt gga gac Leu Leu Phe Phe Ile Phe Ala Ala Leu Gly Val Glu Leu Phe Gly Asp	1730	1735	1740	5232
25	ctg gag tgt gac gag aca cac ccc tgt gag ggc ctg ggc cgt cat gcc Leu Glu Cys Asp Glu Thr His Pro Cys Glu Gly Leu Gly Arg His Ala	1745	1750	1755	5280
30	acc ttt cgg aac ttt ggc atg gcc ttc cta acc ctc ttc cga gtc tcc Thr Phe Arg Asn Phe Gly Met Ala Phe Leu Thr Leu Phe Arg Val Ser	1765	1770	1775	5328
35	aca ggt gac aat tgg aat ggc att atg aag gac acc ctc cgg gac tgt Thr Gly Asp Asn Trp Asn Gly Ile Met Lys Asp Thr Leu Arg Asp Cys	1780	1785	1790	5376
40	gac cag gag tcc acc tgc tac aac acg gtc atc tcg cct atc tac ttt Asp Gln Glu Ser Thr Cys Tyr Asn Thr Val Ile Ser Pro Ile Tyr Phe	1795	1800	1805	5424
45	gtg tcc ttc gtg ctg acg gcc cag ttc gtg cta gtc aac gtg gtg atc Val Ser Phe Val Leu Thr Ala Gln Phe Val Leu Val Asn Val Val Ile	1810	1815	1820	5472
50	gcc gtg ctg atg aag cac ctg gag gag agc aac aag gag gcc aag gag Ala Val Leu Met Lys His Leu Glu Glu Ser Asn Lys Glu Ala Lys Glu	1825	1830	1835	5520
55	gag gcc gag cta gag gct gag ctg gag ctg gag atg aag acc ctc agc Glu Ala Glu Leu Glu Ala Glu Leu Glu Leu Glu Met Lys Thr Leu Ser	1845	1850	1855	5568
60	ccc cag ccc cac tcg cca ctg ggc agc ccc ttc ctc tgg cct ggg gtc Pro Gln Pro His Ser Pro Leu Gly Ser Pro Phe Leu Trp Pro Gly Val	1860	1865	1870	5616
65	gag ggc ccc gac agc ccc gac agc ccc aag cct ggg gct ctg cac cca Glu Gly Pro Asp Ser Pro Asp Ser Pro Lys Pro Gly Ala Leu His Pro	1875	1880	1885	5664
70	gcg gcc cac gcg aga tca gcc tcc cac ttt tcc ctg gag cac ccc acg Ala Ala His Ala Arg Ser Ala Ser His Phe Ser Leu Glu His Pro Thr	1890	1895	1900	5712
75	atg cag ccc cac ccc acg gag ctg cca gga cca gac tta ctg act gtg Met Gln Pro His Pro Thr Glu Leu Pro Gly Pro Asp Leu Leu Thr Val	1905	1910	1915	5760
80	cgg aag tct ggg gtc agc cga acg cac tct ctg ccc aat gac agc tac Arg Lys Ser Gly Val Ser Arg Thr His Ser Leu Pro Asn Asp Ser Tyr				5808

	1925	1930	1935	
5	atg tgt cgg cat ggg agc act gcc gag ggg ccc ctg gga cac agg ggc Met Cys Arg His Gly Ser Thr Ala Glu Gly Pro Leu Gly His Arg Gly 1940 1945 1950	5856		
10	tgg ggg ctc ccc aaa gct cag tca ggc tcc gtc ttg tcc gtt cac tcc Trp Gly Leu Pro Lys Ala Gln Ser Gly Ser Val Leu Ser Val His Ser 1955 1960 1965	5904		
15	cag cca gca gat acc agc tac atc ctg cag ctt ccc aaa gat gca cct Gln Pro Ala Asp Thr Ser Tyr Ile Leu Gln Leu Pro Lys Asp Ala Pro 1970 1975 1980	5952		
20	cat ctg ctc cag ccc cac agc gcc cca acc tgg ggc acc atc ccc aaa His Leu Leu Gln Pro His Ser Ala Pro Thr Trp Gly Thr Ile Pro Lys 1985 1990 2000	6000		
25	ctg ccc cca cca gga cgc tcc cct ttg gct cag agg cca ctc agg cgc Leu Pro Pro Pro Gly Arg Ser Pro Leu Ala Gln Arg Pro Leu Arg Arg 2005 2010 2015	6048		
30	cag gca gca ata agg act gac tcc ttg gac gtt cag ggt ctg ggc agc Gln Ala Ala Ile Arg Thr Asp Ser Leu Asp Val Gln Gly Leu Gly Ser 2020 2025 2030	6096		
35	cgg gaa gac ctg ctg gca gag gtg agt ggg ccc tcc ccg ccc ctg gcc Arg Glu Asp Leu Leu Ala Glu Val Ser Gly Pro Ser Pro Pro Leu Ala 2035 2040 2045	6144		
40	cgg gcc tac tct ttc tgg ggc cag tca agt acc cag gca cag cag cac Arg Ala Tyr Ser Phe Trp Gly Gln Ser Ser Thr Gln Ala Gln Gln His 2050 2055 2060	6192		
45	tcc cgc agc cac agc aag atc tcc aag cac atg acc ccg cca gcc cct Ser Arg Ser His Ser Lys Ile Ser Lys His Met Thr Pro Pro Ala Pro 2065 2070 2075 2080	6240		
50	tgc cca ggc cca gaa ccc aac tgg ggc aag ggc cct cca gag acc aga Cys Pro Gly Pro Glu Pro Asn Trp Gly Lys Gly Pro Pro Glu Thr Arg 2085 2090 2095	6288		
55	agc agc tta gag ttg gac acg gag ctg agc tgg att tca gga gac ctc Ser Ser Leu Glu Leu Asp Thr Glu Leu Ser Trp Ile Ser Gly Asp Leu 2100 2105 2110	6336		
60	ctg ccc cct ggc ggc cag gag gag ccc cca tcc cca cgg gac ctg aag Leu Pro Pro Gly Gly Gln Glu Glu Pro Pro Ser Pro Arg Asp Leu Lys 2115 2120 2125	6384		
65	aag tgc tac agc gtg gag gcc cag agc tgc cag cgc cgg cct acg tcc Lys Cys Tyr Ser Val Glu Ala Gln Ser Cys Gln Arg Arg Pro Thr Ser 2130 2135 2140	6432		
70	tgg ctg gat gag cag agg aga cac tct atc gcc gtc agc tgc ctg gac Trp Leu Asp Glu Gln Arg Arg His Ser Ile Ala Val Ser Cys Leu Asp 2145 2150 2155 2160	6480		
75	agc ggc tcc caa ccc cac ctg ggc aca gac ccc tct aac ctt ggg ggc Ser Gly Ser Gln Pro His Leu Gly Thr Asp Pro Ser Asn Leu Gly Gly 2165 2170 2175	6528		
80	cag cct ctt ggg ggg cct ggg agc cgg ccc aag aaa aaa ctc agc ccg Gln Pro Leu Gly Gly Pro Gly Ser Arg Pro Lys Lys Lys Leu Ser Pro 2180 2185 2190 2195	6576		

	2180	2185	2190	
5	cct agt atc acc ata gac ccc ccc gag agc caa ggt cct cgg acc ccg Pro Ser Ile Thr Ile Asp Pro Pro Glu Ser Gln Gly Pro Arg Thr Pro 2195 2200 2205	6624		
10	ccc agc cct ggt atc tgc ctc cgg agg agg gct ccg tcc agc gac tcc Pro Ser Pro Gly Ile Cys Leu Arg Arg Arg Ala Pro Ser Ser Asp Ser 2210 2215 2220	6672		
15	aag gat ccc ttg gcc tct ggc ccc cct gac agc atg gct gcc tcg ccc Lys Asp Pro Leu Ala Ser Gly Pro Pro Asp Ser Met Ala Ala Ser Pro 2225 2230 2235 2240	6720		
20	tcc cca aag aaa gat gtg ctg agt ctc tcc ggt tta tcc tct gac cca Ser Pro Lys Lys Asp Val Leu Ser Leu Ser Gly Leu Ser Ser Asp Pro 2245 2250 2255	6768		
25	gca gac ctg gac ccc Ala Asp Leu Asp Pro 2260	6783		
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45	ggg ccg ggg tca gca gaa aag gac ccg ggc agc gcg gac tcc gag gcg Gly Pro Gly Ser Ala Glu Lys Asp Pro Gly Ser Ala Asp Ser Glu Ala 35 40 45	144		
50	gag ggg ctg ccg tac ccg gcg ctg gcc ccg gtg gtt ttc ttc tac ttg Glu Gly Leu Pro Tyr Pro Ala Leu Ala Pro Val Val Phe Phe Tyr Leu 50 55 60	192		
55	agc cag gac agc cgc ccg cgg agc tgg tgt ctc cgc acg gtc tgt aac Ser Gln Asp Ser Arg Pro Arg Ser Trp Cys Leu Arg Thr Val Cys Asn 65 70 75 80	240		
60	ccc tgg ttt gag cgc atc agc atg ttg gtc atc ctt ctc aac tgc gtg Pro Trp Phe Glu Arg Ile Ser Met Leu Val Ile Leu Leu Asn Cys Val 85 90 95	288		
65	acc ctg ggc atg ttc cgg cca tgc gag gac atc gcc tgt gac tcc cag Thr Leu Gly Met Phe Arg Pro Cys Glu Asp Ile Ala Cys Asp Ser Gln 100 105 110	336		
70	cgc tgc cgg atc ctg cag gcc ttt gat gac ttc atc ttt gcc ttc ttt Arg Cys Arg Ile Leu Gln Ala Phe Asp Asp Phe Ile Phe Ala Phe Phe 115 120 125	384		

	gcc	gtg	gag	atg	gtg	gtg	aag	atg	gtg	gcc	tgg	ggc	atc	ttt	ggg	aaa	432
	Ala	Val	Glu	Met	Val	Val	Lys	Met	Val	Ala	Leu	Gly	Ile	Phe	Gly	Lys	
	130						135					140					
5	aag	tgt	tac	ctg	gga	gac	act	tgg	aac	cgg	ctt	gac	ttt	ttc	atc	gtc	480
	Lys	Cys	Tyr	Leu	Gly	Asp	Thr	Trp	Asn	Arg	Leu	Asp	Phe	Phe	Ile	Val	
	145					150					155					160	
10	atc	gca	ggg	atg	ctg	gag	tac	tgg	ctg	gac	ctg	cag	aac	gtc	agc	ttc	528
	Ile	Ala	Gly	Met	Leu	Glu	Tyr	Ser	Leu	Asp	Leu	Gln	Asn	Val	Ser	Phe	
					165					170					175		
15	tca	gct	gtc	agg	aca	gtc	cgt	gtg	ctg	cga	ccg	ctc	agg	gcc	att	aac	576
	Ser	Ala	Val	Arg	Thr	Val	Arg	Val	Leu	Arg	Pro	Leu	Arg	Ala	Ile	Asn	
				180					185					190			
20	cgg	gtg	ccc	agc	atg	cgc	atc	ctt	gtc	acg	tgg	ctg	ctg	gat	acg	ctg	624
	Arg	Val	Pro	Ser	Met	Arg	Ile	Leu	Val	Thr	Leu	Leu	Leu	Asp	Thr	Leu	
			195					200					205				
25	ccc	atg	ctg	ggc	aac	gtc	ctg	ctg	ctc	tgc	ttc	ttc	gtc	ttc	ttc	atc	672
	Pro	Met	Leu	Gly	Asn	Val	Leu	Leu	Leu	Cys	Phe	Phe	Val	Phe	Phe	Ile	
	210					215						220					
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	Phe	Gly	Ile	Val	Gly	Val	Gln	Leu	Trp	Ala	Gly	Leu	Leu	Arg	Asn	Arg	
	225					230					235					240	
35	tgc	ttc	cta	cct	gag	aat	ttc	agc	ctc	ccc	ctg	agc	gtg	gac	ctg	gag	768
	Cys	Phe	Leu	Pro	Glu	Asn	Phe	Ser	Leu	Pro	Leu	Ser	Val	Asp	Leu	Glu	
					245					250					255		
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	Arg	Tyr	Tyr	Gln	Thr	Glu	Asn	Glu	Asp	Glu	Ser	Pro	Phe	Ile	Cys	Ser	
				260					265					270			
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	Gln	Pro	Arg	Glu	Asn	Gly	Met	Arg	Ser	Cys	Arg	Ser	Val	Pro	Thr	Leu	
			275					280					285				
50	cgc	ggg	gac	ggg	ggc	ggt	ggc	cca	cct	tgc	ggt	ctg	gac	tat	gag	gcc	912
	Arg	Gly	Asp	Gly	Gly	Gly	Gly	Pro	Pro	Cys	Gly	Leu	Asp	Tyr	Glu	Ala	
	290					295						300					
55	tac	aac	agc	tcc	agc	aac	acc	acc	tgt	gtc	aac	tgg	aac	cag	tac	tac	960
	Tyr	Asn	Ser	Ser	Ser	Asn	Thr	Thr	Cys	Val	Asn	Trp	Asn	Gln	Tyr	Tyr	
	305					310					315					320	
60	acc	aac	tgc	tca	gcg	ggg	gag	cac	aac	ccc	ttc	aag	ggc	gcc	atc	aac	1008
	Thr	Asn	Cys	Ser	Ala	Gly	Glu	His	Asn	Pro	Phe	Lys	Gly	Ala	Ile	Asn	
					325					330					335		
65	ttt	gac	aac	att	ggc	tat	gcc	tgg	atc	gcc	atc	ttc	cag	gtc	atc	acg	1056
	Phe	Asp	Asn	Ile	Gly	Tyr	Ala	Trp	Ile	Ala	Ile	Phe	Gln	Val	Ile	Thr	
				340				345						350			
70	ctg	gag	ggc	tgg	gtc	gac	atc	atg	tac	ttt	gtg	atg	gat	gct	cat	tcc	1104
	Leu	Glu	Gly	Trp	Val	Asp	Ile	Met	Tyr	Phe	Val	Met	Asp	Ala	His	Ser	
			355					360					365				
75	ttc	tac	aat	ttc	atc	tac	ttc	atc	ctc	ctc	atc	atc	gtg	ggc	tcc	ttc	1152
	Phe	Tyr	Asn	Phe	Ile	Tyr	Phe	Ile	Leu	Leu	Ile	Ile	Val	Gly	Ser	Phe	
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	Phe Met Ile Asn Leu Cys Leu Val Val Ile Ala Thr Gln Phe Ser Glu	
	385 390 395 400	
10	acc aag cag cgg gaa agc cag ctg atg cgg gag cag cgt gtg cgg ttc	1248
	Thr Lys Gln Arg Glu Ser Gln Leu Met Arg Glu Gln Arg Val Arg Phe	
	405 410 415	
15	ctg tcc aac gcc agc acc ctg gct agc ttc tct gag ccc ggc agc tgc	1296
	Leu Ser Asn Ala Ser Thr Leu Ala Ser Phe Ser Glu Pro Gly Ser Cys	
	420 425 430	
20	tat gag gag ctg ctc aag tac ctg gtg tac atc ctt cgt aag gca gcc	1344
	Tyr Glu Glu Leu Leu Lys Tyr Leu Val Tyr Ile Leu Arg Lys Ala Ala	
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25	cgc agg ctg gct cag gtc tct cgg gca gca ggt gtg cgg gtt ggg ctg	1392
	Arg Arg Leu Ala Gln Val Ser Arg Ala Ala Gly Val Arg Val Gly Leu	
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30	ctc agc agc cca gca ccc ctc ggg ggc cag gag acc cag ccc agc agc	1440
	Leu Ser Ser Pro Ala Pro Leu Gly Gly Gln Glu Thr Gln Pro Ser Ser	
	465 470 475 480	
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	Ser Cys Ser Arg Ser His Arg Arg Leu Ser Val His His Leu Val His	
	485 490 495	
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	His His His His His His His His Tyr His Leu Gly Asn Gly Thr Leu	
	500 505 510	
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	Arg Ala Pro Arg Ala Ser Pro Glu Ile Gln Asp Arg Asp Ala Asn Gly	
	515 520 525	
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	Ser Arg Arg Leu Met Leu Pro Pro Pro Ser Thr Pro Ala Leu Ser Gly	
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	Ala Pro Pro Gly Gly Ala Glu Ser Val His Ser Phe Tyr His Ala Asp	
	545 550 555 560	
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	Cys His Leu Glu Pro Val Arg Cys Gln Ala Pro Pro Pro Arg Ser Pro	
	565 570 575	
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	Ser Glu Ala Ser Gly Arg Thr Val Gly Ser Gly Lys Val Tyr Pro Thr	
	580 585 590	
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	Val His Thr Ser Pro Pro Pro Glu Thr Leu Lys Glu Lys Ala Leu Val	
	595 600 605	
75	gag gtg gct gcc agc tct ggg ccc cca acc ctc acc agc ctc aac atc	1872
	Glu Val Ala Ala Ser Ser Gly Pro Pro Thr Leu Thr Ser Leu Asn Ile	
	610 615 620	
80	cca ccc ggg ccc tac agc tcc atg cac aag ctg ctg gag aca cag agt	1920
	Pro Pro Gly Pro Tyr Ser Ser Met His Lys Leu Leu Glu Thr Gln Ser	
	625 630 635 640	

	aca ggt gcc tgc caa agc tct tgc aag atc tcc agc cct tgc ttg aaa	1969
	Thr Gly Ala Cys Gln Ser Ser Cys Lys Ile Ser Ser Pro Cys Leu Lys	
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5	gca gac agt gga gcc tgt ggt cca gac agc tgc ccc tac tgt gcc cgg	2016
	Ala Asp Ser Gly Ala Cys Gly Pro Asp Ser Cys Pro Tyr Cys Ala Arg	
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10	gcc ggg gca ggg gag gtg gag ctc gcc gac cgt gaa atg cct gac tca	2064
	Ala Gly Ala Gly Glu Val Glu Leu Ala Asp Arg Glu Met Pro Asp Ser	
	675 680 685	
15	gac agc gag gca gtt tat gag ttc aca cag gat gcc cag cac agc gac	2112
	Asp Ser Glu Ala Val Tyr Glu Phe Thr Gln Asp Ala Gln His Ser Asp	
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	Leu Arg Asp Pro His Ser Arg Arg Gln Arg Ser Leu Gly Pro Asp Ala	
	705 710 715 720	
25	gag ccc agc tct gtg ctg gcc ttc tgg agg cta atc tgt gac acc ttc	2208
	Glu Pro Ser Ser Val Leu Ala Phe Trp Arg Leu Ile Cys Asp Thr Phe	
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	740 745 750	
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	Ile Leu Val Asn Thr Leu Ser Met Gly Ile Glu Tyr His Glu Gln Pro	
	755 760 765	
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	Glu Glu Leu Thr Asn Ala Leu Glu Ile Ser Asn Ile Val Phe Thr Ser	
	770 775 780	
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	Leu Phe Ala Leu Glu Met Leu Leu Lys Leu Leu Val Tyr Gly Pro Phe	
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	Gly Tyr Ile Lys Asn Pro Tyr Asn Ile Phe Asp Gly Val Ile Val Val	
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	Leu Arg Thr Phe Arg Leu Met Arg Val Leu Lys Leu Val Arg Phe Leu	
	835 840 845	
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	Pro Ala Leu Gln Arg Gln Leu Val Val Leu Met Lys Thr Met Asp Asn	
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70	gtg gcc acc ttc tgc atg ctg ctt atg ctc ttc atc ttc atc ttc agc	2640
	Val Ala Thr Phe Cys Met Leu Leu Met Leu Phe Ile Phe Ile Phe Ser	
	865 870 875 880	
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	Ile Leu Gly Met His Leu Phe Gly Cys Lys Phe Ala Ser Glu Arg Asp	
	885 890 895	

	ggg gac acc ctg cca gac cgg aag aat ttt gac tcc ttg ctc tgg gcc	2736
	Gly Asp Thr Leu Pro Asp Arg Lys Asn Phe Asp Ser Leu Leu Trp Ala	
	900 905 910	
5	atc gtc act gtc ttt cag atc ctg acc cag gag gac tgg aac aaa gtc	2784
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	915 920 925	
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	Leu Tyr Asn Gly Met Ala Ser Thr Ser Ser Trp Ala Ala Leu Tyr Phe	
	930 935 940	
15	att gcc ctc atg acc ttc ggc aac tac gtg ctc ttc aat ttg ctg gtc	2880
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	945 950 955 960	
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	Ala Ile Leu Val Glu Gly Phe Gln Ala Glu Gly Asp Ala Asn Lys Ser	
	965 970 975	
25	gaa tca gag ccc gat ttc ttc tca ccc agc ctg gat ggt gat ggg gac	2976
	Glu Ser Glu Pro Asp Phe Phe Ser Pro Ser Leu Asp Gly Asp Gly Asp	
	980 985 990	
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	Arg Lys Lys Cys Leu Ala Leu Val Ser Leu Gly Glu His Pro Glu Leu	
	995 1000 1005	
35	cgg aag agc ctg ctg ccg cct ctc atc atc cac acg gcc gcc aca ccc	3072
	Arg Lys Ser Leu Leu Pro Pro Leu Ile Ile His Thr Ala Ala Thr Pro	
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	Pro Ala Ser Arg Arg Thr Ser Ser Ser Gly Ser Ala Glu Pro Gly Ala	
	1045 1050 1055	
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	Ala His Glu Met Lys Ser Pro Pro Ser Ala Arg Ser Ser Pro His Ser	
	1060 1065 1070	
55	ccc tgg agc gct gca agc agc tgg acc agc agg cgc tcc agc cgg aac	3264
	Pro Trp Ser Ser Ala Ala Ser Ser Trp Thr Ser Arg Arg Ser Ser Arg Asn	
	1075 1080 1085	
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	Glu Ser Ser Glu Glu Glu Arg Ala Ser Pro Ala Gly Ser Asp His Arg	
	1125 1130 1135	
75	cac agg ggg tcc ctg gag cgg gag gcc aag agt tcc ttt gac ctg cca	3456
	His Arg Gly Ser Leu Glu Arg Glu Ala Lys Ser Ser Phe Asp Leu Pro	
	1140 1145 1150	

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	1185 1190 1195 1200	
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	1235 1240 1245	
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	1285 1290 1295	
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	Lys Gly Pro Pro Glu Thr Arg Ser Ser Leu Glu Leu Asp Thr Glu Leu	
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 Gly Pro Gly Ser Ala Glu Lys Asp Pro Gly Ser Ala Asp Ser Glu Ala
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 Pro Trp Phe Glu Arg Ile Ser Met Leu Val Ile Leu Leu Asn Cys Val
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 Thr Leu Gly Met Phe Arg Pro Cys Glu Asp Ile Ala Cys Asp Ser Gln
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35	cga	aag	att	gtg	gac	agc	aag	tac	ttt	ggc	cgg	gga	atc	atg	atc	gcc	2256
	Arg	Lys	Ile	Val	Asp	Ser	Lys	Tyr	Phe	Gly	Arg	Gly	Ile	Met	Ile	Ala	
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40	atc	ctg	gtc	aac	aca	ctc	agc	atg	ggc	atc	gaa	tac	cac	gag	cag	ccc	2304
	Ile	Leu	Val	Asn	Thr	Leu	Ser	Met	Gly	Ile	Glu	Tyr	His	Glu	Gln	Pro	
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45	gag	gag	ctt	acc	aac	gcc	cta	gaa	atc	agc	aac	atc	gtc	ttc	acc	agc	2352
	Glu	Glu	Leu	Thr	Asn	Ala	Leu	Glu	Ile	Ser	Asn	Ile	Val	Phe	Thr	Ser	
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	Leu	Phe	Ala	Leu	Glu	Met	Leu	Leu	Lys	Leu	Leu	Val	Tyr	Gly	Pro	Phe	
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55	ggc	tac	atc	aag	aat	ccc	tac	aac	atc	ttc	gat	ggt	gtc	att	gtg	gtc	2448
	Gly	Tyr	Ile	Lys	Asn	Pro	Tyr	Asn	Ile	Phe	Asp	Gly	Val	Ile	Val	Val	
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	Gly Asp Thr Leu Pro Asp Arg Lys Asn Phe Asp Ser Leu Leu Trp Ala	
	900 905 910	
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	Ile Val Thr Val Phe Gln Ile Leu Thr Gln Glu Asp Trp Asn Lys Val	
	915 920 925	
15	ctc tac aat ggt atg gcc tcc acg tgc tcc tgg gcg gcc cct tat ttc	2832
	Leu Tyr Asn Gly Met Ala Ser Thr Ser Ser Trp Ala Ala Leu Tyr Phe	
	930 935 940	
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	Ile Ala Leu Met Thr Phe Gly Asn Tyr Val Leu Phe Asn Leu Leu Val	
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	gcc att ctg gtg gag ggc ttc cag gcg gag gga gat gcc aac aag tcc	2928
	Ala Ile Leu Val Glu Gly Phe Gln Ala Glu Gly Asp Ala Asn Lys Ser	
	965 970 975	
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	Glu Ser Glu Pro Asp Phe Phe Ser Pro Ser Leu Asp Gly Asp Gly Asp	
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	Arg Lys Lys Cys Leu Ala Leu Val Ser Leu Gly Glu His Pro Glu Leu	
	995 1000 1005	
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	Pro Trp Ser Ala Ala Ser Ser Trp Thr Ser Arg Arg Ser Ser Arg Asn	
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	Arg Arg Ser Leu Leu Ser Gly Glu Gly Gln Glu Ser Gln Asp Glu Glu	
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	Glu Ser Ser Glu Glu Arg Ala Ser Pro Ala Gly Ser Asp His Arg	
	1125 1130 1135	

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	Ser Ala Ser Glu His Gln Asp Cys Asn Gly Lys Ser Ala Ser Gly Arg	
	1170 1175 1180	
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	Arg Gln His Gln Glu Glu Glu Ala Arg Arg Arg Glu Glu Lys Arg	
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	Gly Pro Gly Ser Thr Glu Lys Asp Pro Gly Ser Ala Asp Ser Glu Ala	
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	Glu Gly Leu Pro Tyr Pro Ala Leu Ala Pro Val Val Phe Phe Tyr Leu	
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	Ser Gln Asp Ser Arg Pro Arg Ser Trp Cys Leu Arg Thr Val Cys Asn	
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	Pro Trp Phe Glu Arg Val Ser Met Leu Val Ile Leu Leu Asn Cys Val	
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	Thr Leu Gly Met Phe Arg Pro Cys Glu Asp Ile Ala Cys Asp Ser Gln	
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	Arg Cys Arg Ile Leu Gln Ala Phe Asp Asp Phe Ile Phe Ala Phe Phe	

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50	cag cct cgg gag aat ggc atg aga tcc tgc agg agt gtg ccc aca ctg Gln Pro Arg Glu Asn Gly Met Arg Ser Cys Arg Ser Val Pro Thr Leu 275 280 285			864
55	cgt ggg gaa ggc ggt ggt ggc cca ccc tgc agt ctg gac tat gag acc Arg Gly Glu Gly Gly Gly Gly Pro Pro Cys Ser Leu Asp Tyr Glu Thr 290 295 300			912
60	tat aac agt tcc agc aac acc acc tgt gtc aac tgg aac cag tac tat Tyr Asn Ser Ser Ser Asn Thr Thr Cys Val Asn Trp Asn Gln Tyr Tyr 305 310 315 320			960
65	acc aac tgc tct gcg ggc gag cac aac ccc ttc aaa ggc gcc atc aac Thr Asn Cys Ser Ala Gly Glu His Asn Pro Phe Lys Gly Ala Ile Asn 325 330 335			1008
70	ttt gac aac att ggc tat gcc tgg atc gcc atc ttc cag gtc atc aca Phe Asp Asn Ile Gly Tyr Ala Trp Ile Ala Ile Phe Gln Val Ile Thr 340 345 350			1056
75	ctg gag ggc tgg gtc gac atc atg tac ttc gta atg gac gct cac tcc Leu Glu Gly Trp Val Asp Ile Met Tyr Phe Val Met Asp Ala His Ser 355 360 365			1104
80	ttc tac aac ttc atc tac ttc att ctt ctc atc atc gtg ggc tcc ttc Phe Tyr Asn Phe Ile Tyr Phe Ile Leu Leu Ile Ile Val Gly Ser Phe			1152

	370	375	380	
5	ttc atg atc aac ctg tgc ctg gtg gtg att gcc acg cag ttc tcc gag Phe Met Ile Asn Leu Cys Leu Val Val Ile Ala Thr Gln Phe Ser Glu 385 390 395 400	1200		
10	acc aaa cag cgg gag agt cag ctg atg cgg gag cag cgt gta cga ttc Thr Lys Gln Arg Glu Ser Gln Leu Met Arg Glu Gln Arg Val Arg Phe 405 410 415	1248		
15	ctg tcc aat gct agc acc ctg gca agc ttc tct gag cca ggc agc tgc Leu Ser Asn Ala Ser Thr Leu Ala Ser Phe Ser Glu Pro Gly Ser Cys 420 425 430	1296		
20	tat gag gag cta ctc aag tac ctg gtg tac atc ctc cga aaa gca gcc Tyr Glu Glu Leu Leu Lys Tyr Leu Val Tyr Ile Leu Arg Lys Ala Ala 435 440 445	1344		
25	cga agg ctg gcc cag gtc tct agg gct ata ggc gtg cgg gct ggg ctg Arg Arg Leu Ala Gln Val Ser Arg Ala Ile Gly Val Arg Ala Gly Leu 450 455 460	1392		
30	ctc agc agc cca gtg gcc cgt agt ggg cag gag ccc cag ccc agt ggc Leu Ser Ser Pro Val Ala Arg Ser Gly Gln Glu Pro Gln Pro Ser Gly 465 470 475 480	1440		
35	agc tgc act cgc tca cac cgt cgt ctg tct gtc cac cac ctg gtc cac Ser Cys Thr Arg Ser His Arg Arg Leu Ser Val His His Leu Val His 485 490 495	1488		
40	cac cat cac cac cac cat cac cac tac cac ctg ggt aat ggg acg ctc His His His His His His His His Tyr His Leu Gly Asn Gly Thr Leu 500 505 510	1536		
45	aga gtt ccc cgg gcc agc cca gag atc cag gac agg gat gcc aat ggg Arg Val Pro Arg Ala Ser Pro Glu Ile Gln Asp Arg Asp Ala Asn Gly 515 520 525	1584		
50	tct cgc cgg ctc atg cta cca cca ccc tct aca ccc act ccc tct ggg Ser Arg Arg Leu Met Leu Pro Pro Pro Ser Thr Pro Thr Pro Ser Gly 530 535 540	1632		
55	ggc cct ccg agg ggt gcg gag tct gta cac agc ttc tac cat gct gac Gly Pro Pro Arg Gly Ala Glu Ser Val His Ser Phe Tyr His Ala Asp 545 550 555 560	1680		
60	tgc cac ttg gag cca gtc cgt tgc cag gca ccc cct ccc aga tgc cca Cys His Leu Glu Pro Val Arg Cys Gln Ala Pro Pro Pro Arg Cys Pro 565 570 575	1728		
65	tcg gag gca tct ggt agg act gtg ggt agt ggg aag gtg tac ccc act Ser Glu Ala Ser Gly Arg Thr Val Gly Ser Gly Lys Val Tyr Pro Thr 580 585 590	1776		
70	gtg cat acc agc cct cca cca gag ata ctg aag gat aaa gca cta gtg Val His Thr Ser Pro Pro Pro Glu Ile Leu Lys Asp Lys Ala Leu Val 595 600 605	1824		
75	gag gtg gcc ccc agc cct ggg ccc ccc acc ctc acc agc ttc aac atc Glu Val Ala Pro Ser Pro Gly Pro Pro Thr Leu Thr Ser Phe Asn Ile 610 615 620	1872		
80	cca cct ggg ccc ttc agc tcc atg cac aag ctc ctg gag aca cag agt Pro Pro Gly Pro Phe Ser Ser Met His Lys Leu Leu Glu Thr Gln Ser 625 630 635	1920		

	625		630		635		640	
5	acg gga gcc tgc cat agc tcc tgc aaa atc tcc agc cct tgc tcc aag		645		650		655	1968
	Thr Gly Ala Cys His Ser Ser Cys Lys Ile Ser Ser Pro Cys Ser Lys							
10	gca gac agt gga gcc tgc ggg ccg gac agt tgt ccc tac tgt gcc cgg		660		665		670	2016
	Ala Asp Ser Gly Ala Cys Gly Pro Asp Ser Cys Pro Tyr Cys Ala Arg							
15	aca gga gca gga gag cca gag tcc gct gac cat gtc atg cct gac tca		675		680		685	2064
	Thr Gly Ala Gly Glu Pro Glu Ser Ala Asp His Val Met Pro Asp Ser							
20	gac agc gag gct gtg tat gag ttc aca cag gac gct cag cac agt gac		690		695		700	2112
	Asp Ser Glu Ala Val Tyr Glu Phe Thr Gln Asp Ala Gln His Ser Asp							
25	ctc cgg gat ccc cac agc cgg cgg cga cag cgg agc ctg ggc cca gat		705		710		715	2160
	Leu Arg Asp Pro His Ser Arg Arg Arg Gln Arg Ser Leu Gly Pro Asp							
30	gca gag cct agt tct gtg ctg gct ttc tgg agg ctg atc tgt gac aca		725		730		735	2208
	Ala Glu Pro Ser Ser Val Leu Ala Phe Trp Arg Leu Ile Cys Asp Thr							
35	ttc cgg aag atc gta gat agc aaa tac ttt ggc cgg gga atc atg atc		740		745		750	2256
	Phe Arg Lys Ile Val Asp Ser Lys Tyr Phe Gly Arg Gly Ile Met Ile							
40	gcc atc ctg gtc aat aca ctc agc atg ggc atc gag tac cac gag cag		755		760		765	2304
	Ala Ile Leu Val Asn Thr Leu Ser Met Gly Ile Glu Tyr His Glu Gln							
45	ccc gag gag ctc acc aac gcc ctg gaa atc agc aac atc gtc ttc acc		770		775		780	2352
	Pro Glu Glu Leu Thr Asn Ala Leu Glu Ile Ser Asn Ile Val Phe Thr							
50	agc ctc ttc gcc ttg gag atg ctg ctg aaa ctg ctt gtc tac ggt ccc		785		790		795	2400
	Ser Leu Phe Ala Leu Glu Met Leu Leu Lys Leu Leu Val Tyr Gly Pro							
55	ttt ggc tac att aag aat ccc tac aac atc ttt gat ggt gtc att gtg		805		810		815	2448
	Phe Gly Tyr Ile Lys Asn Pro Tyr Asn Ile Phe Asp Gly Val Ile Val							
60	gtc atc agt gtg tgg gag att gtg ggc cag cag gga ggt ggc ctg tcg		820		825		830	2496
	Val Ile Ser Val Trp Glu Ile Val Gly Gln Gln Gly Gly Gly Leu Ser							
65	gtg ctg cgg acc ttc cgc ctg atg cgg gtg ctg aag ctg gtg cgc ttc		835		840		845	2544
	Val Leu Arg Thr Phe Arg Leu Met Arg Val Leu Lys Leu Val Arg Phe							
70	ctg ccg gcc ctg cag cgc cag ctc gtg gtg ctc atg aag acc atg gac		850		855		860	2592
	Leu Pro Ala Leu Gln Arg Gln Leu Val Val Leu Met Lys Thr Met Asp							
75	aac gtg gcc acc ttc tgc atg ctc ctc atg ctg ttc atc ttc atc ttc		865		870		875	2640
	Asn Val Ala Thr Phe Cys Met Leu Leu Met Leu Phe Ile Phe Ile Phe							
80	agc atc ctg ggc atg cat ctc ttt ggt tgc aag ttc gca tct gaa cgg							2688
	Ser Ile Leu Gly Met His Leu Phe Gly Cys Lys Phe Ala Ser Glu Arg							

	825										890										895										
5	gat	ggg	gac	acg	ttg	cca	gac	cgg	aag	aat	ttc	gac	tcc	ctg	ctc	tgg															2736
	Asp	Gly	Asp	Thr	Leu	Pro	Asp	Arg	Lys	Asn	Phe	Asp	Ser	Leu	Leu	Trp															
				900					905					910																	
10	gcc	atc	gtc	act	gtc	ttt	cag	att	ctg	act	cag	gaa	gac	tgg	aat	aaa															2784
	Ala	Ile	Val	Thr	Val	Phe	Gln	Ile	Leu	Thr	Gln	Glu	Asp	Trp	Asn	Lys															
				915				920					925																		
15	gtc	ctc	tac	aac	ggc	atg	gcc	tcc	aca	tcg	tct	tgg	gct	gct	ctt	tac															2832
	Val	Leu	Tyr	Asn	Gly	Met	Ala	Ser	Thr	Ser	Ser	Trp	Ala	Ala	Leu	Tyr															
				930			935					940																			
20	ttc	atc	gcc	ctc	atg	act	ttt	ggc	aac	tat	gtg	ctc	ttt	aac	ctg	ctg															2880
	Phe	Ile	Ala	Leu	Met	Thr	Phe	Gly	Asn	Tyr	Val	Leu	Phe	Asn	Leu	Leu															
						950					955					960															
25	gtg	gcc	att	ctt	gtg	gaa	gga	ttc	cag	gca	gag	gga	gat	gcc	acc	aag															2928
	Val	Ala	Ile	Leu	Val	Glu	Gly	Phe	Gln	Ala	Glu	Gly	Asp	Ala	Thr	Lys															
					965				970						975																
30	tct	gag	tca	gag	cct	gat	ttc	ttt	tcg	ccc	agt	gtg	gat	ggt	gat	ggg															2976
	Ser	Glu	Ser	Glu	Pro	Asp	Phe	Phe	Ser	Pro	Ser	Val	Asp	Gly	Asp	Gly															
				980				985						990																	
35	gac	aga	aag	aag	cgc	ttg	gcc	ctg	gtg	gct	ttg	gga	gaa	cac	gcg	gaa															3024
	Asp	Arg	Lys	Lys	Arg	Leu	Ala	Leu	Val	Ala	Leu	Gly	Glu	His	Ala	Glu															
				995			1000					1005																			
40	cta	cga	aag	agc	ctt	ttg	cca	ccc	ctc	atc	atc	cat	acg	gct	gcg	aca															3072
	Leu	Arg	Lys	Ser	Leu	Leu	Pro	Pro	Leu	Ile	Ile	His	Thr	Ala	Ala	Thr															
				1010			1015					1020																			
45	cca	atg	tca	cac	ccc	aag	agc	tcc	agc	aca	ggt	gtg	ggg	gaa	gca	ctg															3120
	Pro	Met	Ser	His	Pro	Lys	Ser	Ser	Ser	Thr	Gly	Val	Gly	Glu	Ala	Leu															
						1030				1035					1040																
50	ggc	tct	ggc	tct	cga	cgt	acc	agt	agc	agt	ggg	tcc	gct	gag	cct	gga															3168
	Gly	Ser	Gly	Ser	Arg	Arg	Thr	Ser	Ser	Ser	Gly	Ser	Ala	Glu	Pro	Gly															
					1045			1050						1055																	
55	gct	gcc	cac	cat	gag	atg	aaa	tgt	ccg	cca	agt	gcc	cgc	agc	tcc	ccg															3216
	Ala	Ala	His	His	Glu	Met	Lys	Cys	Pro	Pro	Ser	Ala	Arg	Ser	Ser	Pro															
				1060				1065					1070																		
60	cac	agt	ccc	tgg	agt	gcg	gca	agc	agc	tgg	acc	agc	agg	cgc	tcc	agc															3264
	His	Ser	Pro	Trp	Ser	Ala	Ala	Ser	Ser	Trp	Thr	Ser	Arg	Arg	Ser	Ser															
				1075			1080					1085																			
65	agg	aac	agc	ctg	ggc	cgg	gcc	ccc	agc	cta	aag	cgg	agg	agc	ccg	agc															3312
	Arg	Asn	Ser	Leu	Gly	Arg	Ala	Pro	Ser	Leu	Lys	Arg	Arg	Ser	Pro	Ser															
				1090			1095					1100																			
70	ggg	gag	cgg	agg	tcc	ctg	ctg	tct	gga	gag	ggc	cag	gag	agt	cag	gat															3360
	Gly	Glu	Arg	Arg	Ser	Leu	Leu	Ser	Gly	Glu	Gly	Gln	Glu	Ser	Gln	Asp															
						1110			1115						1120																
75	gag	gag	gaa	agt	tca	gaa	gag	gac	cgg	gcc	agc	cca	gca	ggc	agt	gac															3408
	Glu	Glu	Glu	Ser	Ser	Glu	Glu	Asp	Arg	Ala	Ser	Pro	Ala	Gly	Ser	Asp															
						1125			1130					1135																	
80	cat	cgc	cac	agg	ggt	tcc	ttg	gaa	cgt	gag	gcc	aag	agt	tcc	ttt	gac															3456
	His	Arg	His	Arg	Gly	Ser	Leu	Glu	Arg	Glu	Ala	Lys	Ser	Ser	Phe	Asp															

	1140	1145	1150	
5	ctg cct gac act ctg cag gtg ccg ggg ctg cac cgc aca gcc agc ggc Leu Pro Asp Thr Leu Gln Val Pro Gly Leu His Arg Thr Ala Ser Gly	3504		
	1155	1160	1165	
10	cgg agc tct gcc tct gag cac caa gac tgt aat ggc aag tcg gct tca Arg Ser Ser Ala Ser Glu His Gln Asp Cys Asn Gly Lys Ser Ala Ser	3552		
	1170	1175	1180	
15	ggg cgt ttg gcc cgc acc ctg agg act gat gac ccc caa ctg gat ggc Gly Arg Leu Ala Arg Thr Leu Arg Thr Asp Asp Pro Gln Leu Asp Gly	3600		
	1185	1190	1195	1200
20	gat gat gac aat gat gag gga aat ctg agc aaa ggc gaa cgc ata caa Asp Asp Asp Asn Asp Glu Gly Asn Leu Ser Lys Gly Glu Arg Ile Gln	3648		
	1205	1210	1215	
25	gcc tgg gtc aga tcc cgg ctt cct gcc tgt tgc cga gag cga gat tcc Ala Trp Val Arg Ser Arg Leu Pro Ala Cys Cys Arg Glu Arg Asp Ser	3696		
	1220	1225	1230	
30	tgg tcg gcc tat atc ttt cct cct cag tca agg ttt cgt ctc ctg tgt Trp Ser Ala Tyr Ile Phe Pro Pro Gln Ser Arg Phe Arg Leu Leu Cys	3744		
	1235	1240	1245	
35	cac cgg atc atc acc cac aag atg ttt gac cat gtg gtc ctc gtc atc His Arg Ile Ile Thr His Lys Met Phe Asp His Val Val Leu Val Ile	3792		
	1250	1255	1260	
40	atc ttc ctc aac tgt atc acc atc gct atg gag cgc ccc aaa att gac Ile Phe Leu Asn Cys Ile Thr Ile Ala Met Glu Arg Pro Lys Ile Asp	3840		
	1265	1270	1275	1280
45	ccc cac agc gct gag cgc atc ttc ctg acc ctc tcc aac tac atc ttc Pro His Ser Ala Glu Arg Ile Phe Leu Thr Leu Ser Asn Tyr Ile Phe	3888		
	1285	1290	1295	
50	acg gca gtc ttt cta gct gaa atg aca gtg aag gtg gtg gca ctg ggc Thr Ala Val Phe Leu Ala Glu Met Thr Val Lys Val Val Ala Leu Gly	3936		
	1300	1305	1310	
55	tgg tgc ttt ggg gag cag gcc tac ctg cgc agc agc tgg aat gtg ctg Trp Cys Phe Gly Glu Gln Ala Tyr Leu Arg Ser Ser Trp Asn Val Leu	3984		
	1315	1320	1325	
60	gac ggc ttg ctg gtg ctc atc tcc gtc atc gac atc ctg gtc tcc atg Asp Gly Leu Leu Val Leu Ile Ser Val Ile Asp Ile Leu Val Ser Met	4032		
	1330	1335	1340	
65	gtc tcc gac agc ggc acc aag atc ctt ggc atg ctg agg gtg ctg cgg Val Ser Asp Ser Gly Thr Lys Ile Leu Gly Met Leu Arg Val Leu Arg	4080		
	1345	1350	1355	1360
70	ctg ctg cgg acc ctg cgt cca ctc agg gtc atc agc cgg gcc cag gga Leu Leu Arg Thr Leu Arg Pro Leu Arg Val Ile Ser Arg Ala Gln Gly	4128		
	1365	1370	1375	
75	ctg aag ctg gtg gta gag act ctg atg tca tcc ctc aaa ccc att ggc Leu Lys Leu Val Val Glu Thr Leu Met Ser Ser Leu Lys Pro Ile Gly	4176		
	1380	1385	1390	
80	aac att gtg gtc att tgc tgt gcc ttc ttc atc att ttt gga att ctc Asn Ile Val Val Ile Cys Cys Ala Phe Phe Ile Ile Phe Gly Ile Leu	4224		

	1395	1400	1405	
5	ggg gtg cag ctc ttc aaa ggg aag ttc ttc gtg tgt cag ggt gag gac Gly Val Gln Leu Phe Lys Gly Lys Phe Phe Val Cys Gln Gly Glu Asp 1410 1415 1420	4272		
10	acc agg aac atc act aac aaa tcc gac tgc gct gag gcc agc tac cga Thr Arg Asn Ile Thr Asn Lys Ser Asp Cys Ala Glu Ala Ser Tyr Arg 1425 1430 1435 1440	4320		
	tgg gtc cgg cac aag tac aac ttt gac aac ctg gcc cag gct ctg atg Trp Val Arg His Lys Tyr Asn Phe Asp Asn Leu Gly Gln Ala Leu Met 1445 1450 1455	4368		
15	tcc ctg ttt gtg ctg gcc tcc aag gat ggt tgg gtt gac atc atg tat Ser Leu Phe Val Leu Ala Ser Lys Asp Gly Trp Val Asp Ile Met Tyr 1460 1465 1470	4416		
20	gat ggg ctg gat gct gtg ggt gtg gat cag cag ccc atc atg aac cac Asp Gly Leu Asp Ala Val Gly Val Asp Gln Gln Pro Ile Met Asn His 1475 1480 1485	4464		
25	aac ccc tgg atg ctg cta tac ttc atc tcc ttc ctc ctc atc gtg gcc Asn Pro Trp Met Leu Leu Tyr Phe Ile Ser Phe Leu Leu Ile Val Ala 1490 1495 1500	4512		
30	ttc ttt gtc ctg aac atg ttt gtg gcc gtg gtg gtg gag aac ttc cat Phe Phe Val Leu Asn Met Phe Val Gly Val Val Val Glu Asn Phe His 1505 1510 1515 1520	4560		
	aag tgc aga cag cac cag gag gag gag gag gcc agg cgg cgt gag gag Lys Cys Arg Gln His Gln Glu Glu Glu Glu Ala Arg Arg Arg Glu Glu 1525 1530 1535	4608		
35	aag cga cta cgg agg ctg gag aaa aag aga agg agt aag gag aag cag Lys Arg Leu Arg Arg Leu Glu Lys Lys Arg Arg Ser Lys Glu Lys Gln 1540 1545 1550	4656		
40	atg gcc gaa gcc cag tgc aag ccc tac tac tct gac tac tcg aga ttc Met Ala Glu Ala Gln Cys Lys Pro Tyr Tyr Ser Asp Tyr Ser Arg Phe 1555 1560 1565	4704		
45	cgg ctc ctt gtc cac cac ctg tgt acc agc cac tac ctg gac ctc ttc Arg Leu Leu Val His His Leu Cys Thr Ser His Tyr Leu Asp Leu Phe 1570 1575 1580	4752		
50	atc act ggt gtc atc ggg ctg aac gtg gtc act atg gcc atg gaa cat Ile Thr Gly Val Ile Gly Leu Asn Val Val Thr Met Ala Met Glu His 1585 1590 1595 1600	4800		
	tac cag cag ccc cag atc ctg gac gag gct ctg aag atc tgc aat tac Tyr Gln Gln Pro Gln Ile Leu Asp Glu Ala Leu Lys Ile Cys Asn Tyr 1605 1610 1615	4848		
55	atc ttt acc gtc atc ttt gtc ttt gag tca gtt ttc aaa ctt gtg gcc Ile Phe Thr Val Ile Phe Val Phe Glu Ser Val Phe Lys Leu Val Ala 1620 1625 1630	4896		
60	ttt ggc ttc cgc cgt ttc ttc cag gac agg tgg aac cag ctg gac ctg Phe Gly Phe Arg Arg Phe Phe Gln Asp Arg Trp Asn Gln Leu Asp Leu 1635 1640 1645	4944		
	gct att gtg ctt ctg tcc atc atg gcc atc aca ctg gag gag att gag Ala Ile Val Leu Leu Ser Ile Met Gly Ile Thr Leu Glu Glu Ile Glu 1650 1655 1660	4992		

	1650	1655	1660	
5	gtc aat ctg tct ctg ccc atc aac ccc acc atc atc cgt atc atg agg Val Asn Leu Ser Leu Pro Ile Asn Pro Thr Ile Ile Arg Ile Met Arg 1665 1670 1675 1680	5040		
10	gtg ctc cgc att gct cga gtt ctg aag ctg ttg aag atg gct gtg ggc Val Leu Arg Ile Ala Arg Val Leu Lys Leu Leu Lys Met Ala Val Gly 1685 1690 1695	5088		
15	atg cgg gca ctg ctg cac acg gtg atg cag gcc ctg ccc cag gtg ggg Met Arg Ala Leu Leu His Thr Val Met Gln Ala Leu Pro Gln Val Gly 1700 1705 1710	5136		
20	aac ctg gga ctt ctc ttc atg tta ttg ttt ttc atc ttt gca gct ctg Asn Leu Gly Leu Leu Phe Met Leu Leu Phe Phe Ile Phe Ala Ala Leu 1715 1720 1725	5184		
25	ggc gtg gag ctc ttt gga gac ctg gag tgt gat gag aca cac cct tgt Gly Val Glu Leu Phe Gly Asp Leu Glu Cys Asp Glu Thr His Pro Cys 1730 1735 1740	5232		
30	gag ggc ttg ggt cgg cat gcc acc ttt agg aac ttt ggt atg gcc ttt Glu Gly Leu Gly Arg His Ala Thr Phe Arg Asn Phe Gly Met Ala Phe 1745 1750 1755 1760	5280		
35	ctg acc ctc ttc cga gtc tcc act ggt gac aac tgg aat ggt att atg Leu Thr Leu Phe Arg Val Ser Thr Gly Asp Asn Trp Asn Gly Ile Met 1765 1770 1775	5328		
40	aag gac acc ctc cgg gac tgt gac cag gag tcc acc tgc tac aac act Lys Asp Thr Leu Arg Asp Cys Asp Gln Glu Ser Thr Cys Tyr Asn Thr 1780 1785 1790	5376		
45	gtc atc tcc cct atc tac ttt gtg tcc ttc gtg ctg acg gcc cag ttt Val Ile Ser Pro Ile Tyr Phe Val Ser Phe Val Leu Thr Ala Gln Phe 1795 1800 1805	5424		
50	gtg ctg gtc aac gtg gtc ata gct gtg ctg atg aag cac ctg gaa gaa Val Leu Val Asn Val Val Ile Ala Val Leu Met Lys His Leu Glu Glu 1810 1815 1820	5472		
55	agc aac aaa gag gcc aag gag gag gcc gag ctc gag gcc gag ctg gag Ser Asn Lys Glu Ala Lys Glu Glu Ala Glu Leu Glu Ala Glu Leu Glu 1825 1830 1835 1840	5520		
60	ctg gag atg aag acg ctc agc ccg cag ccc cac tcc ccg ctg ggc agc Leu Glu Met Lys Thr Leu Ser Pro Gln Pro His Ser Pro Leu Gly Ser 1845 1850 1855	5568		
65	ccc ttc ctc tgg ccc ggg gtg gag ggt gtc aac agt act gac agc cct Pro Phe Leu Trp Pro Gly Val Glu Gly Val Asn Ser Thr Asp Ser Pro 1860 1865 1870	5616		
70	aag cct ggg gct cca cac acc act gcc cac att gga gca gcc tct ggc Lys Pro Gly Ala Pro His Thr Thr Ala His Ile Gly Ala Ala Ser Gly 1875 1880 1885	5664		
75	ttc tcc ctt gag cac ccc acg atg gta ccc cac ccc gag gag gtg cca Phe Ser Leu Glu His Pro Thr Met Val Pro His Pro Glu Glu Val Pro 1890 1895 1900	5712		
80	gtc ccc cta gga cca gac ctg ctg act gtg agg aag tct ggt gtc agc Val Pro Leu Gly Pro Asp Leu Leu Thr Val Arg Lys Ser Gly Val Ser 1905 1910 1915	5760		

	1905	1910	1915	1920	
5	cgg acg cac tct ctg Arg Thr His Ser	ccc aat gac agc Pro Asn Asp Ser	tac atg tgc cgc Tyr Met Cys Arg	aat ggg agc Asn Gly Ser	5808
		1925	1930	1935	
10	act gct gag aga tcc Thr Ala Glu Arg	cta gga cac agg Ser Leu Gly His	ggc tgg ggg ctc Gly Trp Gly Leu	ccc aaa gcc Pro Lys Ala	5856
		1940	1945	1950	
	cag tca ggc tcc atc Gln Ser Gly Ser	ttg tcc gtt cac Ile Leu Ser Val	tcc caa cca gca Ser Gln Pro Ala	gac acc agc Asp Thr Ser	5904
		1955	1960	1965	
15	tgc atc cta cag ctt Cys Ile Leu Gln	ccc aaa gat gtg Pro Lys Asp Val	cac tat ctg ctc His Tyr Leu Leu	cag cct cat Gln Pro His	5952
		1970	1975	1980	
20	ggg gct ccc acc tgg Gly Ala Pro Thr	ggc gcc atc cct Gly Ala Ile Pro	aaa cta ccc cca Lys Leu Pro Pro	cct ggc cgc Pro Gly Arg	6000
		1990	1995	2000	
25	tcc cct ctg gct cag Ser Pro Leu Ala	agg cct ctc agg Gln Arg Pro Leu	cgc cag gca gca Arg Gln Ala Ala	ata agg act Ile Arg Thr	6048
		2005	2010	2015	
30	gac tcc ctg gat gtg Asp Ser Leu Asp	gtg cag ggc ctg Val Gln Gly Leu	ggt agc cgg gaa Gly Ser Arg Glu	gac ctg ttg tca Asp Leu Leu Ser	6096
		2020	2025	2030	
	gag gtg agt ggg ccc Glu Val Ser Gly	tcc tgc cct ctg Pro Ser Cys Pro	acc cgg tcc tca Thr Arg Ser Ser	tcc ttc tgg Ser Phe Trp	6144
		2035	2040	2045	
35	ggc ggg tgc agc atc Gly Gly Ser Ser	cag gtg cag cag Gln Val Gln Gln	cgt tcc ggc atc Arg Ser Gly Ile	cag agc aaa Gln Ser Lys	6192
		2050	2055	2060	
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		2085	2090	2095	
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	2165	2170	2175	
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	Arg Val Pro Ser Met Arg Ile Leu Val Thr Leu Leu Leu Asp Thr Leu	
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	Arg Ser Phe Thr Gln Leu Asn Asp Leu Ser Gly Ala Gly Gly Arg Gln	
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	Gly Pro Gly Ser Thr Glu Lys Asp Pro Gly Ser Ala Asp Ser Glu Ala	
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	Glu Gly Leu Pro Tyr Pro Ala Leu Ala Pro Val Val Phe Phe Tyr Leu	
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	Ser Gln Asp Ser Arg Pro Arg Ser Trp Cys Leu Arg Thr Val Cys Asn	
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	Pro Trp Phe Glu Arg Val Ser Met Leu Val Ile Leu Leu Asn Cys Val	
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	Thr Leu Gly Met Phe Arg Pro Cys Glu Asp Ile Ala Cys Asp Ser Gln	
	100 105 110	

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	Ala Val Glu Met Val Val Lys Met Val Ala Leu Gly Ile Phe Gly Lys	
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	Arg Val Pro Ser Met Arg Ile Leu Val Thr Leu Leu Leu Asp Thr Leu	
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	Cys Phe Leu Pro Glu Asn Phe Ser Leu Pro Leu Ser Val Asp Leu Glu	
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	305 310 315 320	
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	Phe Tyr Asn Phe Ile Tyr Phe Ile Leu Leu Ile Ile Val Gly Ser Phe	
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	Phe Met Ile Asn Leu Cys Leu Val Val Ile Ala Thr Gln Phe Ser Glu	
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	Thr Lys Gln Arg Glu Ser Gln Leu Met Arg Glu Gln Arg Val Arg Phe	
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	Leu Ser Asn Ala Ser Thr Leu Ala Ser Phe Ser Glu Pro Gly Ser Cys	
	420 425 430	
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	Tyr Glu Glu Leu Leu Lys Tyr Leu Val Tyr Ile Leu Arg Lys Ala Ala	
	435 440 445	
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	Leu Lys Leu Val Val Glu Thr Leu Met Ser Ser Leu Lys Pro Ile Gly	
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		530					535					540					
50	ggc	cct	ccg	agg	ggt	gcg	gag	tct	gta	cac	agc	ttc	tac	cat	gct	gac	1680
	Gly	Pro	Pro	Arg	Gly	Ala	Glu	Ser	Val	His	Ser	Phe	Tyr	His	Ala	Asp	
		545				550					555					560	
55	tgc	cac	ttg	gag	cca	gtc	cgt	tgc	cag	gca	ccc	cct	ccc	aga	tgc	cca	1728
	Cys	His	Leu	Glu	Pro	Val	Arg	Cys	Gln	Ala	Pro	Pro	Pro	Arg	Cys	Pro	
					565					570					575		
	tcg	gag	gca	tct	ggt	agg	act	gtg	ggt	agt	ggg	aag	gtg	tac	ccc	act	1776
	Ser	Glu	Ala	Ser	Gly	Arg	Thr	Val	Gly	Ser	Gly	Lys	Val	Tyr	Pro	Thr	
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60	gtg	cat	acc	agc	cct	cca	cca	gag	ata	ctg	aag	gat	aaa	gca	cta	gtg	1824
	Val	His	Thr	Ser	Pro	Pro	Pro	Glu	Ile	Leu	Lys	Asp	Lys	Ala	Leu	Val	
			595					600					605				
	gag	gtg	gcc	ccc	agc	cct	ggg	ccc	ccc	acc	ctc	acc	agc	ttc	aac	atc	1872

	Glu	Val	Ala	Pro	Ser	Pro	Gly	Pro	Pro	Thr	Leu	Thr	Ser	Phe	Asn	Ile	
	610						615					620					
5	cca	cct	ggg	ccc	ttc	agc	tcc	atg	cac	aag	ctc	ctg	gag	aca	cag	agt	1920
	Pro	Pro	Gly	Pro	Phe	Ser	Ser	Met	His	Lys	Leu	Leu	Glu	Thr	Gln	Ser	
	625					630					635					640	
10	acg	gga	gcc	tgc	cat	agc	tcc	tgc	aaa	atc	tcc	agc	cct	tgc	tcc	aag	1968
	Thr	Gly	Ala	Cys	His	Ser	Ser	Cys	Lys	Ile	Ser	Ser	Pro	Cys	Ser	Lys	
					645					650					655		
15	gca	gac	agt	gga	gcc	tgc	ggg	cgg	gac	agt	tgt	ccc	tac	tgt	gcc	cgg	2016
	Ala	Asp	Ser	Gly	Ala	Cys	Gly	Pro	Asp	Ser	Cys	Pro	Tyr	Cys	Ala	Arg	
				660					665					670			
	aca	gga	gca	gga	gag	cca	gag	tcc	gct	gac	cat	gtc	atg	cct	gac	tca	2064
	Thr	Gly	Ala	Gly	Glu	Pro	Glu	Ser	Ala	Asp	His	Val	Met	Pro	Asp	Ser	
				675				680					685				
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	Asp	Ser	Glu	Ala	Val	Tyr	Glu	Phe	Thr	Gln	Asp	Ala	Gln	His	Ser	Asp	
		690					695					700					
25	ctc	cgg	gat	ccc	cac	agc	cgg	cgg	cga	cag	cgg	agc	ctg	ggc	cca	gat	2160
	Leu	Arg	Asp	Pro	His	Ser	Arg	Arg	Arg	Gln	Arg	Ser	Leu	Gly	Pro	Asp	
	705					710					715					720	
30	gca	gag	cct	agt	tct	gtg	ctg	gct	ttc	tgg	agg	ctg	atc	tgt	gac	aca	2208
	Ala	Glu	Pro	Ser	Ser	Val	Leu	Ala	Phe	Trp	Arg	Leu	Ile	Cys	Asp	Thr	
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	Phe	Arg	Lys	Ile	Val	Asp	Ser	Lys	Tyr	Phe	Gly	Arg	Gly	Ile	Met	Ile	
				740					745					750			
	gcc	atc	ctg	gtc	aat	aca	ctc	agc	atg	ggc	atc	gag	tac	cac	gag	cag	2304
	Ala	Ile	Leu	Val	Asn	Thr	Leu	Ser	Met	Gly	Ile	Glu	Tyr	His	Glu	Gln	
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40	ccc	gag	gag	ctc	acc	aac	gcc	ctg	gaa	atc	agc	aac	atc	gtc	ttc	acc	2352
	Pro	Glu	Glu	Leu	Thr	Asn	Ala	Leu	Glu	Ile	Ser	Asn	Ile	Val	Phe	Thr	
		770					775					780					
45	agc	ctc	ttc	gcc	ttg	gag	atg	ctg	ctg	aaa	ctg	ctt	gtc	tac	ggc	ccc	2400
	Ser	Leu	Phe	Ala	Leu	Glu	Met	Leu	Leu	Lys	Leu	Leu	Val	Tyr	Gly	Pro	
		785				790					795					800	
50	ttt	ggc	tac	att	aag	aat	ccc	tac	aac	atc	ttt	gat	ggc	gtc	att	gtg	2448
	Phe	Gly	Tyr	Ile	Lys	Asn	Pro	Tyr	Asn	Ile	Phe	Asp	Gly	Val	Ile	Val	
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55	gtc	atc	agt	gtg	tgg	gag	att	gtg	ggc	cag	cag	gga	ggc	ggc	ctg	tcg	2496
	Val	Ile	Ser	Val	Trp	Glu	Ile	Val	Gly	Gln	Gln	Gly	Gly	Gly	Leu	Ser	
				820					825					830			
	gtg	ctg	cgg	acc	ttc	cgc	ctg	atg	cgg	gtg	ctg	aag	ctg	gtg	cgc	ttc	2544
	Val	Leu	Arg	Thr	Phe	Arg	Leu	Met	Arg	Val	Leu	Lys	Leu	Val	Arg	Phe	
			835					840					845				
60	ctg	cgg	gcc	ctg	cag	cgc	cag	ctc	gtg	gtg	ctc	atg	aag	acc	atg	gac	2592
	Leu	Pro	Ala	Leu	Gln	Arg	Gln	Leu	Val	Val	Leu	Met	Lys	Thr	Met	Asp	
		850					855					860					
	aac	gtg	gcc	acc	ttc	tgc	atg	ctc	ctc	atg	ctg	ttc	atc	ttc	atc	ttc	2640

	Asn	Val	Ala	Thr	Phe	Cys	Met	Leu	Leu	Met	Leu	Phe	Ile	Phe	Ile	Phe	
	865					870					875					880	
5	agc	atc	ctg	ggc	atg	cat	ctc	ttt	ggt	tgc	aag	ttc	gca	tct	gaa	cgg	2688
	Ser	Ile	Leu	Gly	Met	His	Leu	Phe	Gly	Cys	Lys	Phe	Ala	Ser	Glu	Arg	
					885					890					895		
10	gat	ggg	gac	acg	ttg	cca	gac	cgg	aag	aat	ttc	gac	tcc	ctg	ctc	tgg	2736
	Asp	Gly	Asp	Thr	Leu	Pro	Asp	Arg	Lys	Asn	Phe	Asp	Ser	Leu	Leu	Trp	
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15	gcc	atc	gtc	act	gtc	ttt	cag	att	ctg	act	cag	gaa	gac	tgg	aat	aaa	2784
	Ala	Ile	Val	Thr	Val	Phe	Gln	Ile	Leu	Thr	Gln	Glu	Asp	Trp	Asn	Lys	
			915					920					925				
20	gtc	ctc	tac	aac	ggc	atg	gcc	tcc	aca	tcg	tct	tgg	gct	gct	ctt	tac	2832
	Val	Leu	Tyr	Asn	Gly	Met	Ala	Ser	Thr	Ser	Ser	Trp	Ala	Ala	Leu	Tyr	
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25	ttc	atc	gcc	ctc	atg	act	ttt	ggc	aac	tat	gtg	ctc	ttt	aac	ctg	ctg	2880
	Phe	Ile	Ala	Leu	Met	Thr	Phe	Gly	Asn	Tyr	Val	Leu	Phe	Asn	Leu	Leu	
	945					950					955				960		
30	gtg	gcc	att	ctt	gtg	gaa	gga	ttc	cag	gca	gag	gga	gat	gcc	acc	aag	2928
	Val	Ala	Ile	Leu	Val	Glu	Gly	Phe	Gln	Ala	Glu	Gly	Asp	Ala	Thr	Lys	
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	Ser	Glu	Ser	Glu	Pro	Asp	Phe	Phe	Ser	Pro	Ser	Val	Asp	Gly	Asp	Gly	
				980					985					990			
40	gac	aga	aag	aag	cgc	ttg	gcc	ctg	gtg	gct	ttg	gga	gaa	cac	gcg	gaa	3024
	Asp	Arg	Lys	Lys	Arg	Leu	Ala	Leu	Val	Ala	Leu	Gly	Glu	His	Ala	Glu	
			995					1000					1005				
45	cta	cga	aag	agc	ctt	ttg	cca	ccc	ctc	atc	atc	cat	acg	gct	gcg	aca	3072
	Leu	Arg	Lys	Ser	Leu	Leu	Pro	Pro	Leu	Ile	Ile	His	Thr	Ala	Ala	Thr	
		1010					1015					1020					
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	Pro	Met	Ser	His	Pro	Lys	Ser	Ser	Ser	Thr	Gly	Val	Gly	Glu	Ala	Leu	
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55	ggc	tct	ggc	tct	cga	cgt	acc	agt	agc	agt	ggg	tcc	gct	gag	cct	gga	3168
	Gly	Ser	Gly	Ser	Arg	Arg	Thr	Ser	Ser	Ser	Gly	Ser	Ala	Glu	Pro	Gly	
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	Ala	Ala	His	His	Glu	Met	Lys	Cys	Pro	Pro	Ser	Ala	Arg	Ser	Ser	Pro	
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	His	Ser	Pro	Trp	Ser	Ala	Ala	Ser	Ser	Trp	Thr	Ser	Arg	Arg	Ser	Ser	
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	Arg	Asn	Ser	Leu	Gly	Arg	Ala	Pro	Ser	Leu	Lys	Arg	Arg	Ser	Pro	Ser	
		1090					1095						1100				
75	ggg	gag	cgg	agg	tcc	ctg	ctg	tct	gga	gag	ggc	cag	gag	agt	cag	gat	3360
	Gly	Glu	Arg	Arg	Ser	Leu	Leu	Ser	Gly	Glu	Gly	Gln	Glu	Ser	Gln	Asp	
		1105				1110					1115				1120		
80	gag	gag	gaa	agt	tca	gaa	gag	gac	cgg	gcc	agc	cca	gca	ggc	agt	gac	3408

	Glu	Glu	Glu	Ser	Ser	Glu	Glu	Asp	Arg	Ala	Ser	Pro	Ala	Gly	Ser	Asp	
					1125					1130					1135		
5	cat	cgc	cac	agg	ggt	tcc	ttg	gaa	cgt	gag	gcc	aag	agt	tcc	ttt	gac	3456
	His	Arg	His	Arg	Gly	Ser	Leu	Glu	Arg	Glu	Ala	Lys	Ser	Ser	Phe	Asp	
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10	ctg	cct	gac	act	ctg	cag	gtg	ccg	ggg	ctg	cac	cgc	aca	gcc	agc	ggc	3504
	Leu	Pro	Asp	Thr	Leu	Gln	Val	Pro	Gly	Leu	His	Arg	Thr	Ala	Ser	Gly	
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	Arg	Ser	Ser	Ala	Ser	Glu	His	Gln	Asp	Cys	Asn	Gly	Lys	Ser	Ala	Ser	
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	Gly	Arg	Leu	Ala	Arg	Thr	Leu	Arg	Thr	Asp	Asp	Pro	Gln	Leu	Asp	Gly	
					1185			1190			1195					1200	
25	gat	gat	gac	aat	gat	gag	gga	aat	ctg	agc	aaa	ggg	gaa	cgc	ata	caa	3648
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30	gcc	tgg	gtc	aga	tcc	cgg	ctt	cct	gcc	tgt	tgc	cga	gag	cga	gat	tcc	3696
	Ala	Trp	Val	Arg	Ser	Arg	Leu	Pro	Ala	Cys	Cys	Arg	Glu	Arg	Asp	Ser	
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	Trp	Ser	Ala	Tyr	Ile	Phe	Pro	Pro	Gln	Ser	Arg	Phe	Arg	Leu	Leu	Cys	
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	His	Arg	Ile	Ile	Thr	His	Lys	Met	Phe	Asp	His	Val	Val	Leu	Val	Ile	
					1250			1255				1260					
45	atc	ttc	ctc	aac	tgt	atc	acc	atc	gct	atg	gag	cgc	ccc	aaa	att	gac	3840
	Ile	Phe	Leu	Asn	Cys	Ile	Thr	Ile	Ala	Met	Glu	Arg	Pro	Lys	Ile	Asp	
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50	ccc	cac	agc	gct	gag	cgc	atc	ttc	ctg	acc	ctc	tcc	aac	tac	atc	ttc	3888
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55	acg	gca	gtc	ttt	cta	gct	gaa	atg	aca	gtg	aag	gtg	gtg	gca	ctg	ggc	3936
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	Trp	Cys	Phe	Gly	Glu	Gln	Ala	Tyr	Leu	Arg	Ser	Ser	Trp	Asn	Val	Leu	
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65	gac	ggc	ttg	ctg	gtg	ctc	atc	tcc	gtc	atc	gac	atc	ctg	gtc	tcc	atg	4032
	Asp	Gly	Leu	Leu	Val	Leu	Ile	Ser	Val	Ile	Asp	Ile	Leu	Val	Ser	Met	
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	Leu	Leu	Arg	Thr	Leu	Arg	Pro	Leu	Arg	Val	Ile	Ser	Arg	Ala	Gln	Gly	
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5	aac	att	gtg	gtc	att	tgc	tgt	gcc	ttc	ttc	atc	att	ttt	gga	att	ctc	4224
	Asn	Ile	Val	Val	Ile	Cys	Cys	Ala	Phe	Phe	Ile	Ile	Phe	Gly	Ile	Leu	
			1395					1400					1405				
10	ggg	gtg	cag	ctc	ttc	aaa	ggg	aag	ttc	ttc	gtg	tgt	cag	ggt	gag	gac	4272
	Gly	Val	Gln	Leu	Phe	Lys	Gly	Lys	Phe	Phe	Val	Cys	Gln	Gly	Glu	Asp	
		1410					1415					1420					
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	Thr	Arg	Asn	Ile	Thr	Asn	Lys	Ser	Asp	Cys	Ala	Glu	Ala	Ser	Tyr	Arg	
	1425					1430					1435					1440	
	tgg	gtc	cgg	cac	aag	tac	aac	ttt	gac	aac	ctg	ggc	cag	gct	ctg	atg	4368
	Trp	Val	Arg	His	Lys	Tyr	Asn	Phe	Asp	Asn	Leu	Gly	Gln	Ala	Leu	Met	
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	Ser	Leu	Phe	Val	Leu	Ala	Ser	Lys	Asp	Gly	Trp	Val	Asp	Ile	Met	Tyr	
			1460						1465					1470			
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	Asp	Gly	Leu	Asp	Ala	Val	Gly	Val	Asp	Gln	Gln	Pro	Ile	Met	Asn	His	
		1475					1480						1485				
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	Asn	Pro	Trp	Met	Leu	Leu	Tyr	Phe	Ile	Ser	Phe	Leu	Leu	Ile	Val	Ala	
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	1505				1510					1515					1520		
	aag	tgc	aga	cag	cac	cag	gag	gag	gag	gag	gag	gcg	agg	cgg	cgt	gag	4608
	Lys	Cys	Arg	Gln	His	Gln	Glu	Glu	Glu	Glu	Ala	Arg	Arg	Arg	Glu	Glu	
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40	aag	cga	cta	cgg	agg	ctg	gag	aaa	aag	aga	agg	aaa	gcc	cag	tgc	aag	4656
	Lys	Arg	Leu	Arg	Arg	Leu	Glu	Lys	Lys	Arg	Arg	Lys	Ala	Gln	Cys	Lys	
			1540					1545					1550				
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	Pro	Tyr	Tyr	Ser	Asp	Tyr	Ser	Arg	Phe	Arg	Leu	Leu	Val	His	His	Leu	
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	Cys	Thr	Ser	His	Tyr	Leu	Asp	Leu	Phe	Ile	Thr	Gly	Val	Ile	Gly	Leu	
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55	aac	gtg	gtc	act	atg	gcc	atg	gaa	cat	tac	cag	cag	ccc	cag	atc	ctg	4800
	Asn	Val	Val	Thr	Met	Ala	Met	Glu	His	Tyr	Gln	Gln	Pro	Gln	Ile	Leu	
	1585					1590					1595				1600		
	gac	gag	gct	ctg	aag	atc	tgc	aat	tac	atc	ttt	acc	gtc	atc	ttt	gtc	4848
	Asp	Glu	Ala	Leu	Lys	Ile	Cys	Asn	Tyr	Ile	Phe	Thr	Val	Ile	Phe	Val	
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	Phe	Glu	Ser	Val	Phe	Lys	Leu	Val	Ala	Phe	Gly	Phe	Arg	Arg	Phe	Phe	
			1620					1625					1630				
	cag	gac	agg	tgg	aac	cag	ctg	gac	ctg	gct	att	gtg	ctt	ctg	tcc	atc	4944

	Gln Asp Arg Trp Asn Gln Leu Asp Leu Ala Ile Val Leu Leu Ser Ile	
	1635 1640 1645	
5	atg ggc atc aca ctg gag gag att gag gtc aat ctg tcg ctg ccc atc Met Gly Ile Thr Leu Glu Glu Ile Glu Val Asn Leu Ser Leu Pro Ile	4992
	1650 1655 1660	
10	aac ccc acc atc atc cgt atc atg agg gtg ctc cgc att gct cga gtt Asn Pro Thr Ile Ile Arg Ile Met Arg Val Leu Arg Ile Ala Arg Val	5040
	1665 1670 1675 1680	
15	ctg aag ctg ttg aag atg gct gtg ggc atg cgg gca ctg ctg cac acg Leu Lys Leu Leu Lys Met Ala Val Gly Met Arg Ala Leu Leu His Thr	5088
	1685 1690 1695	
	gtg atg cag gcc ctg ccc cag gtg ggg aac ctg gga ctt ctc ttc atg Val Met Gln Ala Leu Pro Gln Val Gly Asn Leu Gly Leu Leu Phe Met	5136
	1700 1705 1710	
20	tta ttg ttt ttc atc ttt gca gct ctg ggc gtg gag ctc ttt gga gac Leu Leu Phe Phe Ile Phe Ala Ala Leu Gly Val Glu Leu Phe Gly Asp	5184
	1715 1720 1725	
25	ctg gag tgt gat gag aca cac cct tgt gag ggc ttg ggt cgg cat gcc Leu Glu Cys Asp Glu Thr His Pro Cys Glu Gly Leu Gly Arg His Ala	5232
	1730 1735 1740	
30	acc ttt agg aac ttt ggt atg gcc ttt ctg acc ctc ttc cga gtc tcc Thr Phe Arg Asn Phe Gly Met Ala Phe Leu Thr Leu Phe Arg Val Ser	5280
	1745 1750 1755 1760	
35	act ggt gac aac tgg aat ggt att atg aag gac acc ctc cgg gac tgt Thr Gly Asp Asn Trp Asn Gly Ile Met Lys Asp Thr Leu Arg Asp Cys	5328
	1765 1770 1775	
	gac cag gag tcc acc tgc tac aac act gtc atc tcc cct atc tac ttt Asp Gln Glu Ser Thr Cys Tyr Asn Thr Val Ile Ser Pro Ile Tyr Phe	5376
	1780 1785 1790	
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	1795 1800 1805	
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	1860 1865 1870	
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	1875 1880 1885	
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	Leu	Thr	Val	Arg	Lys	Ser	Gly	Val	Ser	Arg	Thr	His	Ser	Leu	Pro	Asn	
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	Asp	Ser	Tyr	Met	Cys	Arg	Asn	Gly	Ser	Thr	Ala	Glu	Arg	Ser	Leu	Gly	
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	His	Arg	Gly	Trp	Gly	Leu	Pro	Lys	Ala	Gln	Ser	Gly	Ser	Ile	Leu	Ser	
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	Asp	Val	His	Tyr	Leu	Leu	Gln	Pro	His	Gly	Ala	Pro	Thr	Trp	Gly	Ala	
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	Ile	Pro	Lys	Leu	Pro	Pro	Pro	Gly	Arg	Ser	Pro	Leu	Ala	Gln	Arg	Pro	
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	Leu	Arg	Arg	Gln	Ala	Ala	Ile	Arg	Thr	Asp	Ser	Leu	Asp	Val	Gln	Gly	
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	Glu	Thr	Arg	Ser	Ser	Leu	Glu	Leu	Asp	Thr	Glu	Leu	Ser	Trp	Ile	Ser	
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	Gly	Asp	Leu	Leu	Pro	Ser	Ser	Gln	Glu	Glu	Pro	Leu	Phe	Pro	Arg	Asp	
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	Leu	Lys	Lys	Cys	Tyr	Ser	Val	Glu	Thr	Gln	Ser	Cys	Arg	Arg	Arg	Pro	
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	Gly	Phe	Trp	Leu	Asp	Glu	Gln	Arg	Arg	His	Ser	Ile	Ala	Val	Ser	Cys	
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 2165 2170 2175

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 Ser Pro Pro Ser Ile Ser Ile Asp Pro Pro Glu Ser Gln Gly Ser Arg
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 Pro Pro Cys Ser Pro Gly Val Cys Leu Arg Arg Arg Ala Pro Ala Ser
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 Arg Gly Ala Gly Thr Arg Gly Gly Gly Phe Glu Leu Gly Val Ser
 35 40 45

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 Pro Ser Glu Ser Pro Ala Ala Glu Arg Cys Ala Glu Leu Gly Ala Asp
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 Glu Glu Gln Arg Val Pro Tyr Pro Ala Leu Ala Ala Thr Val Phe Phe
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 Cys Leu Gly Gln Thr Thr Arg Pro Arg Ser Trp Cys Leu Arg Leu Val
 85 90 95

tgc aac cca tgg ttc gag cac gtg agc atg ctg gta atc atg ctc aac 336
 Cys Asn Pro Trp Phe Glu His Val Ser Met Leu Val Ile Met Leu Asn

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	ttt ttt gcg gtg gag atg gtc atc aag atg gtg gcc ttg ggg ctg ttc Phe Phe Ala Val Glu Met Val Ile Lys Met Val Ala Leu Gly Leu Phe 145 150 155 160			480
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	aac cag ctg gac ctg gcc atc gtg ctg ctg tca ctc atg ggc atc acg Asn Gln Leu Asp Leu Ala Ile Val Leu Leu Ser Leu Met Gly Ile Thr 1685 1690 1695			5088
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	Cys Leu Gly Gln Thr Thr Arg Pro Arg Ser Trp Cys Leu Arg Leu Val	
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	Cys Asn Pro Trp Phe Glu His Val Ser Met Leu Val Ile Met Leu Asn	
	100 105 110	
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	Cys Val Thr Leu Gly Met Phe Arg Pro Cys Glu Asp Val Glu Cys Gly	
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	195 200 205	
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	Tyr Val Met Asp Ala His Ser Phe Tyr Asn Phe Ile Tyr Phe Ile Leu	
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	Leu Ile Ile Val Gly Ser Phe Phe Met Ile Asn Leu Cys Leu Val Val	
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	Pro Gly Arg Gly Pro Pro Asp Ala Glu Ser Val His Ser Ile Tyr His	
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	Arg His Arg Gly His Gly Pro Leu Ser Leu Asn Ser Pro Asp Pro Tyr	
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	Pro Pro Gly Leu Glu Glu Pro Leu Glu Gly Thr Asn Pro Asp Val Pro	
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Lys Met Ala

40

INTERNATIONAL SEARCH REPORT

Intern Application No

PCT/US 98/23161

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12N15/12 C07K14/705 C07K16/28 C12N5/10 G01N33/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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Y	see abstract; claims 1-10	3,19
X	NOONEY JM (REPRINT) ET AL: "Identifying neuronal non-L Ca ²⁺ channels - more than stamp collecting?" TRENDS IN PHARMACOLOGICAL SCIENCES, 10-1997, 18, 363-371, XP002093637 see page 369, right-hand column - page 370, right-hand column	1,2, 10-16, 20-22



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

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X document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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Date of the actual completion of the international search

16 February 1999

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Gurdjian, D

INTERNATIONAL SEARCH REPORT

Intern 1st Application No
PCT/US 98/23161

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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Y	& EMBL DATABASE Accession number q18840 WILSON R. ET AL. 1996 see the whole document ---	3,19
A	WO 93 04083 A (SALK INST BIOTECH IND) 4 March 1993 see abstract; claims 1-39 ---	1-22
P,X	PEREZ-REYES E ET AL: "Molecular characterization of a neuronal low-voltage-activated T-type calcium channel 'see comments!'" NATURE, FEB 26 1998, 391 (6670) P896-900, XP002093639 ENGLAND see the whole document ---	1-15, 20-22
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INTERNATIONAL SEARCH REPORT

Information on patent family members

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PCT/US 98/23161

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